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☐ 1. 20040052799. 30 Dec 02. 18 Mar 04. Nucleic acid and amino acid sequences relating to Helicobacter pylori for diagnostics and therapeutics. Smith, Douglas, et al. 424/184.1; A61K039/00 A61K039/38.

☐ 2. 20040002440. 19 Feb 03. 01 Jan 04. Novel diagnostic agents of chronic or persistent chlamydial diseases and uses thereof. Timms, Peter, et al. 514/2; 435/6 435/7.32 514/44 A61K038/16 A61K048/00 G01N033/554 G01N033/569 C12Q001/68.

☐ 3. 20020015678. 09 Feb 99. 07 Feb 02. DIRECT ADSORPTION SCINTILLATION ASSAY FOR MEASURING ENZYME ACTIVITY AND ASSAYING BIOCHEMICAL PROCESSES. YUAN, ZHENGYU, et al. 424/1.11; 422/102 435/4 435/7.1 435/7.2 436/501 436/518 436/537 A61K051/00 C12Q001/00 G01N033/53 G01N033/567 G01N033/543 G01N033/542 G01N033/566.

☐ 4. 6534278. 03 Mar 00; 18 Mar 03. Screening for antibiotics. Rothstein; David M.. 435/7.2; 435/18 435/183 435/19 435/24 435/243 435/32 435/471 435/6 435/69.1 435/69.8 435/7.1 435/7.32 435/7.4 536/24.3 536/24.32. G01N033/53.

☐ 5. 6310193. 30 Sep 97; 30 Oct 01. MurC from Streptococcus pneumoniae. Black; Michael Terence, et al. 536/23.5; 435/252.3 435/252.33 435/320.1 435/325 435/362 435/365 435/367 435/69.3 536/23.6 536/23.7. C07H021/04 C12N015/09 C12N005/00 C12N001/20.

☐ 6. 5891621. 24 Sep 97; 06 Apr 99. Metabolic pathway assay. Chabin; Renee M., et al. 435/4; 435/15 435/16 435/18 435/21 435/23 435/24 435/32 435/7.91. C12Q001/00 C12Q001/37 C12Q001/18 C12Q001/48.

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☐ 11. WO2003027139A. Novel crystallized recombinant polypeptides from Staphylococcus aureus, Streptococcus pneumoniae and Helicobacter pylori and which are involved in membrane biosynthesis,

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☐ 1. 20040052799. 30 Dec 02. 18 Mar 04. Nucleic acid and amino acid sequences relating to Helicobacter pylori for diagnostics and therapeutics. Smith, Douglas, et al. 424/184.1; A61K039/00 A61K039/38.

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L2: Entry 1 of 64 File: PGPB Apr 29, 2004

PGPUB-DOCUMENT-NUMBER: 20040082002
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20040082002 A1

TITLE: 37 staphylococcus aureus genes and polypeptides

PUBLICATION-DATE: April 29, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Choi, Gil H.	Rockville	MD	US	

APPL-NO: 10/ 712713 [PALM]
DATE FILED: November 14, 2003

RELATED-US-APPL-DATA:
Application 10/712713 is a continuation-of US application 10/084205, filed February 28, 2002, PENDING
Application 10/084205 is a continuation-in-part-of US application PC/T/US00/23773, filed August 31, 2000, PENDING
Application is a non-provisional-of-provisional application 60/151933, filed September 1, 1999,

INT-CL: [07] C07 K 14/31, C12 Q 1/68, C07 H 21/04, C12 N 1/21

US-CL-PUBLISHED: 435/006; 435/069.1, 435/320.1, 435/252.3, 530/350, 536/023.7
US-CL-CURRENT: 435/6; 435/252.3, 435/320.1, 435/69.1, 530/350, 536/23.7

ABSTRACT:

The present invention relates to novel genes from S. aureus and the polypeptides they encode. Also provided as are vectors, host cells, antibodies and recombinant methods for producing the same. The invention further relates to screening methods for identifying agonists and antagonists of S. aureus polypeptide activity. The invention additionally relates to diagnostic methods for detecting Staphylococcus nucleic acids, polypeptides and antibodies in a biological sample. The present invention further relates to novel vaccines for the prevention or attenuation of infection by Staphylococcus.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present application is a continuation of and claims priority under 35 U.S.C. .sctn. 120 to U.S. patent application Ser. No. 10/084,205, filed Feb. 28, 2002, which is a continuation-in-part of and claims priority under 35 U.S.C. .sctn. 120 to International Application No. PCT/US00/23773, filed Aug. 31, 2000 (published by the International Bureau in the English language on Mar. 8, 2001 as International Publication No. WO 01/16292A2), which claims benefit under 35 U.S.C. .sctn. 119(e) to U.S. Provisional Application No. 60/151,933, filed Sep. 1, 1999, each of which is hereby incorporated by reference in its entirety.

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☐ 48. 6153381. 28 Aug 97; 28 Nov 00. Screening for antibiotics. Rothstein; David M.. 435/6; 435/18 435/183 435/19 435/24 435/243 435/32 435/69.1 435/69.8 435/7.1 435/7.32 435/7.4 536/24.3 536/24.32. C12Q001/68.

☐ 49. 6037123. 13 Sep 96; 14 Mar 00. Methods of screening for compounds active on Staphylococcus aureus target genes. Benton; Bret, et al. 435/6; 435/69.1 436/501 514/2 514/44. C12Q001/68.

☐ 50. 5891621. 24 Sep 97; 06 Apr 99. Metabolic pathway assay. Chabin; Renee M., et al. 435/4; 435/15 435/16 435/18 435/21 435/23 435/24 435/32 435/7.91. C12Q001/00 C12Q001/37 C12Q001/18 C12Q001/48.

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Terms	Documents
L1 same (peptide or polypeptide or protein or amino or sequence or nucleic or nucleotide or polynucleotide or dna or mrna or cdna or c-dna or rna or poly-nucleotide or nuclear)	64

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WEST Search History

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DATE: Thursday, June 17, 2004

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☐	L1	expression near2 vector	28950
☐	L2	L1 same (definition or defined or defining or meant or means or intended or scope)	6527
☐	L3	L2 same (nucleotide or polynucleotide or chromosome or chromosomal or plasmid)	2867
☐	L4	L2 same (nucleotide or polynucleotide or chromosome or chromosomal)	1901
☐	L5	l4 same definition	119
☐	L6	l4 same definition	119
☐	L7	l1 near5 (native or natural or nonforeign or non-foreign or nonheterologous or naturally)	965
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☐	L9	l1 near3 native	205
☐	L10	l1 near3 (nonrecombinant or non-recombinant)	5
☐	L11	naked near3 (dna or rna or nucleotide or polynucleotide or poly-nucleotide or cdna or mrna or m-rna or c-dna or chromosomal or chromosome)	2828
☐	L12	L11 same l1	441

END OF SEARCH HISTORY

First Hit

L3: Entry 3 of 17 File: PGPB Feb 7, 2002

DOCUMENT-IDENTIFIER: US 20020015678 A1
TITLE: DIRECT ADSORPTION SCINTILLATION ASSAY FOR MEASURING ENZYME ACTIVITY AND ASSAYING BIOCHEMICAL PROCESSES

CLAIMS:

What is claimed is:

1) A method for analyzing a sample comprising: a) providing a sample containing one or more molecular species, wherein at least one of the molecular species is capable of stimulating scintillation; b) providing a scintillating material, wherein the surface of the scintillating material adsorbs at least one of the molecular species via a general molecular property-based binding interaction between the molecular species and the scintillating material, and where the scintillating material can be stimulated to scintillate by at least one of the adsorbed molecular species, but is generally not stimulated to scintillate by any molecular species which is not adsorbed; c) measuring the scintillation emitted by the scintillating material.

2) The method of claim 1, wherein the number of molecular species provided is at least two, and where at least one of said molecular species has a presence of, an absence of, or a degree of general molecular property-based binding interaction with with the scintillating material distinct from the remainder of the molecular species.

3) The method of claim 1, wherein the general molecular property-based binding interaction is selected from the group consisting of charge-charge interactions, dipole-charge interactions, dipole-dipole interactions and hydrophobic interactions. interactions.

4) The method of claim 2, wherein the presence of, the absence of, or the degree of general molecular property-based binding interaction with the scintillating material material is due to a chemical or biochemical transformation of one of said molecular molecular species into another of said molecular species, further comprising the step of determining the progress of or degree of completion of the molecular transformation.

5) The method of claim 1, wherein the scintillating material is selected from the group consisting of scintillating plastics and scintillating glasses.

6) The method of claim 1, wherein the scintillating material is a plastic doped with with a scintillant.

7) The method of claim 5, wherein the scintillating plastic is selected from the group consisting of polystyrene doped with at least one scintillating fluor and polyvinyltoluene doped with at least one scintillating fluor.

8) The method of claim 2, wherein at least one of the at least two molecular species species provided is a substrate for an enzyme-catalyzed reaction or a series of enzyme-catalyzed reactions, another of the at least two molecular species is a

product of the enzyme-catalyzed reaction or series of enzyme-catalyzed reactions and and has a presence of, absence of, or degree of general molecular property-based binding affinity for the scintillating material distinct from that of the substrate, substrate, and where the difference in general molecular property-based binding affinity is a result of the enzyme-catalyzed reaction or series of enzyme-catalyzed reactions.

9) The method of claim 8, wherein the general molecular property-based binding affinity is due to the presence of positive charge, the absence of positive charge, the presence of negative charge, the absence of negative charge, the presence of a dipole moment, the absence of a dipole moment, the presence of hydrophobicity, or the absence of hydrophobicity.

10) The method of claim 8, wherein the enzyme catalyzed reaction is selected from the group consisting of kinase catalyzed reactions, lipase catalyzed reactions, phosphatase catalyzed reactions, protease catalyzed reactions, and tRNA transferase catalyzed reactions.

11) The method of claim 8, wherein the enzyme catalyzed reaction is selected from the group consisting of the reaction cascade or any portion thereof for the sequential synthesis of uridinediphosphate-N-acetylmurami- c acid pentapeptide catalyzed by the enzymes MurA, MurB, MurC, MurD, MurE, and MurF.

12) The method of claim 8, wherein the enzyme catalyzed reaction is that catalyzed by MurA.

13) The method of claim 8, wherein the enzyme catalyzed reaction is that catalyzed by MurB.

14) The method of claim 8, wherein the enzyme catalyzed reaction is that catalyzed by MurC.

15) The method of claim 8, wherein the enzyme catalyzed reaction is that catalyzed by MurD.

16) The method of claim 8, wherein the enzyme catalyzed reaction is that catalyzed by MurE.

17) The method of claim 8, wherein the enzyme catalyzed reaction is that catalyzed by MurF.

18) The method of claim 8, wherein the enzyme catalyzed reaction is the reaction cascade for the sequential synthesis of uridinediphosphate-N-ace- tylmuramic acid pentapeptide catalyzed by the enzymes MurA, MurB, MurC, MurD, MurE, and MurF.

19) The method of claim 4, further comprising performing the method on a plurality of samples to effect a high throughput screen.

20) The method of claim 19, wherein the high throughput screen is used to identify compounds which inhibit an enzyme catalyzed reaction selected from the group consisting of the reaction cascade or any portion thereof for the sequential synthesis of uridinediphosphate-N-acetylmuramic acid pentapeptide catalyzed by the enzymes MurA, MurB, MurC, MurD, MurE, and MurF; kinase catalyzed reactions, lipase catalyzed reactions, phosphatase catalyzed reactions, protease catalyzed reactions, and tRNA transferase catalyzed reactions

21) A plate suitable for a direct adsorption binding assay, said plate comprised of a scintillating material and having one or more wells.

22) A plate suitable for a direct adsorption binding assay, said plate comprising

wells coated with a scintillant material.

23) The plate of claim 21, wherein said wells are derivatized such that the walls of of the wells are positively charged.

24) The plate of claim 21, wherein said wells are derivatized such that the walls of of the wells are negatively charged.

25) The plate of claim 21, wherein said wells are derivatized such that the walls of of the wells are hydrophobic.

26) The plate of claim 23, wherein the walls of the wells are derivatized with methyltrioctylammonium bromide.

27) The plate of claim 24, wherein the walls of the wells are derivatized with octadecyl sulfate.

28) The plate of claim 25, where the walls of the wells are derivatized with polylysine-N.sup.68-palmitate.

First Hit Fwd Refs

L3: Entry 4 of 17 File: USPT Mar 18, 2003

US-PAT-NO: 6534278
DOCUMENT-IDENTIFIER: US 6534278 B1
** See image for Certificate of Correction **

TITLE: Screening for antibiotics

DATE-ISSUED: March 18, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Rothstein; David M.	Lexington	MA		

US-CL-CURRENT: 435/7.2; 435/18, 435/183, 435/19, 435/24, 435/243, 435/32, 435/471, 435/6, 435/69.1, 435/69.8, 435/7.1, 435/7.32, 435/7.4, 536/24.3, 536/24.32

CLAIMS:

What is claimed is:

1. A method for identifying a candidate compound that inhibits bacterial growth, the method comprising: providing bactena carrying a gene that encodes, .beta.-actamase; incubating the bacteria with the candidate compound under conditions that enable cell wall biosynthesis, to form a reaction mixture; performing a first assay on the reaction mixture to detect induction of .beta.-lactamase, wherein induction of .beta.-lactamase indicates that the candidate compound is an inhibitor of bacterial growth; obtaining a cell extract containing enzymes, cofactors, and carrier molecules necessary for a particular step or steps of cell wall biosynthesis; supplying a substrate for the step or steps; incubating the candidate compound with the cell extract and the substrate under conditions that enable the step or steps to proceed to form an incubation mixture; and performing a second assay on thee incubation mixture for the substrate and the product normally produced in the step or steps, wherein the production of an amount of product less than that normally produced in the step or steps relative to the amount of substrate indicates the presence of an inhibitor of the step or steps, to thereby identify a candidate compound that inhibits bacterial growth.

2. A method for identifying an inhibitor of a particular step or steps of cell wall biosynthesis, the method comprising: providing bacteria carrying a gene that encodes .beta.-lactamase; incubating the bacteria with a candidate compound under conditions that enable cell wall biosynthesis, to form a reaction mixture; performing a first assay on the reaction mixture to detect induction of .beta.-lactamase to identify an inhibitor of cell wall biosynthesis; obtaining a cell extract containing enzymes, cofactors, and carrier molecules necessary for the particular step or steps; supplying a substrate for the step or steps; incubating the inhibitor with the cell extract and the substrate under conditions that enable the step or steps to proceed; and performing a second assay on the incubation mixture for the substrate and the product normally produced in the step or steps, wherein the production of an amount of product less than that normally produced in the step or steps relative to the amount of substrate indicates the presence of an inhibitor of the step or steps, to thereby identify an inhibitor of a particular step or steps of cell wall biosynthesis.

3. A method of claim 2, wherein the candidate compound is a member of a library of potential inhibitors.

4. A method of claim 2, wherein the first assaying step comprises measuring the optical density of the reaction mixture.

5. A method of claim 2, wherein the first assaying step comprises detecting the binding of antibodies to .beta.-lactamase.

6. A method of claim 2, wherein the cell extract is selected from the group consisting of a whole cell, a cell membrane preparation, and a cytoplasmic extract.

7. A method of claim 2, wherein the substrate is detectably labeled.

8. A method of claim 2, wherein the second assaying step comprises chromatography.

9. A method of claim 2, wherein the step of cell wall biosynthesis is the enolpyruvyl transfer step catalyzed by MurA or MurZ.

10. A method of claim 2, wherein the step of cell wall biosynthesis is the reduction of uridine diphosphate N-acetylenolpyruvylglucosamine catalyzed by MurB.

11. A method of claim 2, wherein the step of cell wall biosynthesis is the addition of L-alanine to uridine diphosphate-N-acetylmuramic acid catalyzed by MurC.

12. A method of claim 2, wherein the step of cell wall biosynthesis is the addition of D-glutamic acid to uridine diphosphate-N-acetylmuramic acid-L-alanine catalyzed by MurD.

13. A method of claim 2, wherein the step of cell wall biosynthesis is the addition of meso-diaminopimelate to uridine diphosphate-N-acetylmuramic acid-dipeptide catalyzed by MurE.

14. A method of claim 2, wherein the step of cell wall biosynthesis is the addition of D-alanyl-D-alanine to uridine diphosphate-N-acetylmuramic acid-tripeptide catalyzed by MurF.

15. A method of claim 2, wherein the step of cell wall biosynthesis is the racemization of L-alanine to D-alanine catalyzed by Ala racemase.

16. A method of claim 2, wherein the step of cell wall biosynthesis is the ligation of two molecules of D-alanine catalyzed by D-Ala:D-Ala ligase.

17. A method of claim 2, wherein the step of cell wall biosynthesis is the synthesis of lipid-linked N-acetylmuramic acid-pentapeptide catalyzed by MraY.

18. A method of claim 2, wherein the step of cell wall biosynthesis is the N-acetylglucosamine transfer step catalyzed by MurG.

19. A method of claim 2, wherein the step of cell wall biosynthesis is septum peptidoglycan synthesis catalyzed by the peptidoglycan transglycosylase-transpeptidase FtsI.

20. A method of claim 2, wherein the step of cell wall biosynthesis is septum peptidoglycan synthesis catalyzed by FtsW.

21. A method of claim 2, wherein the gene tit encodes .beta.-lactamase is from a bacterial species selected from the group of genera consisting of Citrobacter, Enterobacter, Serratia, Pseudomonas, and Proteus.

22. A method of claim 21, wherein the gene is ampC from Citrobacter freundii.

23. A method of claim 2, wherein the gene also includes a reporter gene.

24. A method of claim 23, wherein the reporter gene is lacz.

25. A method of claim 23, wherein the reporter gene is luc.

First Hit Fwd Refs

L3: Entry 5 of 17 File: USPT Oct 30, 2001

DOCUMENT-IDENTIFIER: US 6310193 B1
TITLE: MurC from Streptococcus pneumoniae

Abstract Text (1):
The invention provides MurC polypeptides and DNA (RNA) encoding MurC polypeptides and methods for producing such polypeptides by recombinant techniques. Also provided are methods for utilizing MurC polypeptides to screen for antibacterial compounds.

First Hit Fwd Refs

L3: Entry 6 of 17 File: USPT Apr 6, 1999

DOCUMENT-IDENTIFIER: US 5891621 A
TITLE: Metabolic pathway assay

CLAIMS:

10. The kit according to claim 9 wherein the first enzyme is the gene product of murC, the second enzyme is the gene product of murD, the third enzyme is the gene product of murE, and the fourth enzyme is the gene product of murF.

15. The kit according to claim 14 wherein the first enzyme is the gene product of murA, the second enzyme is the gene product of murB, the third enzyme is the gene product of murC, the fourth enzyme is the gene product of murD, the fifth enzyme is the gene product of MurE, and the sixth enzyme is the product of MurF.

29. The method according to claim 28 wherein the first enzyme is the gene product of murC, the second enzyme is the gene product of murD, the third enzyme is the gene gene product of murE, and the fourth enzyme is the gene product of murF.

35. The method according to claim 34 wherein the first enzyme is the gene product of murA, the second enzyme is the gene product of murB, the third enzyme is the gene product of murC, the fourth enzyme is the gene product of murD, the fifth enzyme is the gene product of murE, and the sixth enzyme is the product of murF.

37. A high-throughput in vitro screening method for detecting a biologically active compound which is comprised of:

(a) combining an enzyme cascade comprising a first enzyme, a second enzyme, a third enzyme, a fourth enzyme, a fifth enzyme and a sixth enzyme, and a labeled substrate for the first enzyme with a compound suspected of having biological activity; wherein:

the first enzyme is the gene product of murA, the second enzyme is the gene product of murB, the third enzyme is the gene product of murC, the fourth enzyme is the gene product of murD, the fifth enzyme is the gene product of murE, and the sixth enzyme is the product of murF; and

(b) measuring the concentrations of the products of the enzymes and comparing to a standard, by absorbing the product of the sixth enzyme onto resin and detecting the amount of label and comparing the amount of label to control.

SRNT

US005891621A

United States Patent [19]

Chabin et al.

[11] **Patent Number: 5,891,621**

[45] **Date of Patent: Apr. 6, 1999**

[54] **METABOLIC PATHWAY ASSAY**

[75] Inventors: **Renee M. Chabin**, Neptune; **David W. Kuo**, Princeton; **John F. O'Connell**, Cranbury; **David L. Pompliano**, Lawrenceville; **Kenny K. Wong**, Edison, all of N.J.

[73] Assignee: **Merck & Co., Inc.**, Rahway, N.J.

[21] Appl. No.: **936,646**

[22] Filed: **Sep. 24, 1997**

Related U.S. Application Data

[60] Provisional application No. 60/027,331 Sep. 30, 1996 and 60/043,249, Apr. 16, 1997.

[51] **Int. Cl.**[6] **C12Q 1/00**; C12Q 1/37; C12Q 1/18; C12Q 1/48

[52] **U.S. Cl.** **435/4**; 435/23; 435/24; 435/32; 435/15; 435/21; 435/7.91; 435/18; 435/16

[58] **Field of Search** 435/4, 16, 23, 435/24, 32, 15, 21, 7.91, 18

[56] **References Cited**

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Pratviel–Sosa, et al., "Effect of various analogues of D–glutamic acid on the D–glutamate–adding enzyme from *Escherichia coli*," FEMS, Microbiol. Letters, vol. 115, pp. 223–228 (1994).

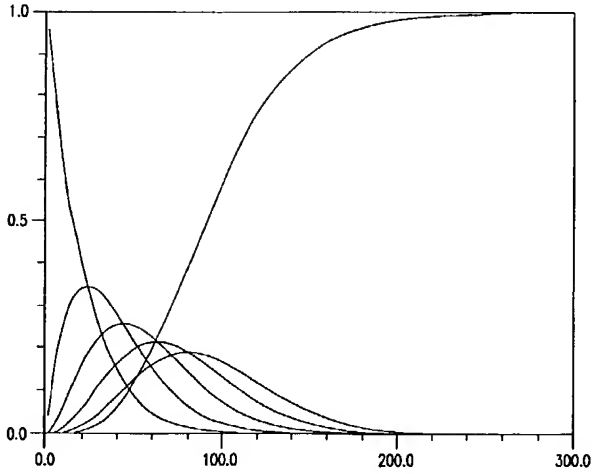
(List continued on next page.)

Primary Examiner—Louise N. Leary
Attorney, Agent, or Firm—Catherine D. Fitch; Melvin Winokur

[57] **ABSTRACT**

An in vitro screening assay which identifies enzyme inhibitors and allows for the simultaneous assay of many enzymes. Enzyme, substrate, co-factor, etc. concentrations are optimized so that inhibitors of any one of the enzymes in the pathway are equally likely to be detected. Necessarily, the flux of substrate through each enzyme should be nearly the same during the assay, i.e., each of the enzyme catalyzed steps must be equally rate-limiting. Preferably, optimal assay conditions are predicted by computer modeling. Further, the pathway conditions are optimized through variation of enzyme, starting substrate, co-substrate and co-factor concentrations. A positive response is initially detected as a change in the amount of the product generated at the end of the enzyme cascade as compared to a standard. A sample producing a positive result can be deconvoluted.

37 Claims, 2 Drawing Sheets



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(19)日本国特許庁(JP)　(12)公開特許公報(A)　(11)特許出願公開番号
特開2002-300888
(P2002-300888A)
(43)公開日　平成14年10月15日(2002.10.15)

(51)Int.Cl.'	識別記号	FI	テーマコード(参考)
C12N 15/09	ZNA	A61K 31/7088	2G045
A61K 31/7088		45/00	4B024
38/43		48/00	4B063
38/55		A61P 1/00	4B064
45/00		7/02	4B065

審査請求 未請求 請求項の数16 OL (全35頁) 最終頁に続く

(21)出願番号　特願2001-381079(P2001-381079)
(62)分割の表示　特願平10-225115の分割
(22)出願日　平成10年7月3日(1998.7.3)

(31)優先権主張番号　60/052720
(32)優先日　平成9年7月3日(1997.7.3)
(33)優先権主張国　米国(US)

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最終頁に続く

(54)【発明の名称】　MurC

(57)【要約】　(修正有)
【課題】　MurCポリペプチドおよびMurCポリペプチドをコードしているポリヌクレオチド、ならびに組換法によるかかるポリペプチドの製造方法を提供する。さらに、抗細菌化合物のスクリーニングのためのMurCポリペプチドの使用方法も提供する。
【解決手段】　特定のアミノ酸配列に対して少なくとも70%の同一性を有するポリペプチド、ならびにアミノ酸配列に対して少なくとも70%の同一性を有するポリペプチドをコードしているポリヌクレオチド配列を含むポリヌクレオチド。

First Hit

L3: Entry 7 of 17 File: JPAB Oct 15, 2002

DOCUMENT-IDENTIFIER: JP 2002300888 A
TITLE: MURC

Abstract Text (1):
PROBLEM TO BE SOLVED: To provide a MurC polypeptide, to provide a polynucleotide encoding the MurC polypeptide, to provide a method for producing the polypeptide by a recombination method, and to provide a method for using the MurC polypeptide for screening an antibacterial compound.

First Hit

L3: Entry 8 of 17 File: JPAB Aug 24, 1999

DOCUMENT-IDENTIFIER: JP 11225773 A
TITLE: MURC

Abstract Text (1):
PROBLEM TO BE SOLVED: To obtain MurC polypeptide and MurC polynucleotide useful e.g. e.g. for screening of new antibiotics and antibacterial therapies.

First Hit

L3: Entry 8 of 17 File: JPAB Aug 24, 1999

PUB-NO: JP411225773A
DOCUMENT-IDENTIFIER: JP 11225773 A
TITLE: MURC

PUBN-DATE: August 24, 1999

INVENTOR-INFORMATION:

NAME	COUNTRY
WALLIS, NICOLA G	
BURNHAM, MARTIN K R	

INT-CL (IPC): C12 N 15/09; A61 K 31/70; A61 K 38/53; A61 K 38/55; A61 K 39/085; A61 K 39/395; A61 K 39/395; A61 K 48/00 ; A61 K 48/00; C07 K 16/40; C12 N 1/21; C12 P 21/02; C12 P 21/08; C12 Q 1/68; G01 N 33/566

First Hit

L3: Entry 7 of 17 File: JPAB Oct 15, 2002

PUB-NO: JP02002300888A
DOCUMENT-IDENTIFIER: JP 2002300888 A
TITLE: MURC

PUBN-DATE: October 15, 2002

INVENTOR-INFORMATION:

NAME	COUNTRY
WALLIS, NICOLA G	
BURNHAM, MARTIN K R	

INT-CL (IPC): C12 N 15/09; A61 K 31/7088; A61 K 38/43; A61 K 38/55; A61 K 45/00; A61 K 48/00; A61 P 1/00; A61 P 7/02; A61 P 9/00; A61 P 11/00; A61 P 13/02; A61 P 13/12; A61 P 17/00; A61 P 17/02; A61 P 19/02; A61 P 19/08; A61 P 21/00; A61 P 25/00; A61 P 27/00; A61 P 29/00; A61 P 31/04; A61 P 39/02; A61 P 43/00; C07 K 14/195; C12 N 1/15; C12 N 1/19; C12 N 1/21; C12 N 5/10; C12 P 21/02; C12 Q 1/02; C12 Q 1/68; G01 N 33/15; G01 N 33/50

First Hit

L3: Entry 9 of 17 File: JPAB Jul 27, 1999

PUB-NO: JP411196876A
DOCUMENT-IDENTIFIER: JP 11196876 A
TITLE: NEW MURC GENE

PUBN-DATE: July 27, 1999

INVENTOR-INFORMATION:

NAME	COUNTRY
KOBAYASHI, MIKI	
YUGAWA, HIDEAKI	
WACHI, MASAAKI	
NAGAI, KAZUO	

INT-CL (IPC): C12 N 15/09; C12 N 9/00

First Hit

L3: Entry 9 of 17 File: JPAB Jul 27, 1999

DOCUMENT-IDENTIFIER: JP 11196876 A
TITLE: NEW MURC GENE

Abstract Text (1):
PROBLEM TO BE SOLVED: To obtain a gene for UDP-N-acetylmuramic acid-alanine ligase gene (murC gene) derived from coryneform bacteria.

Abstract Text (2):
SOLUTION: This invention relates to a DNA fragment obtained by treating a chromosomal DNA derived from coryneform bacteria with the restriction enzymes, MluI and EcoRV, wherein, one end is an MluI site and the other end is an EcoRV site, and including at least a DNA fragment having a size of about 1.9 kb, which is divided by EcoRV into two fragments, one having a size of about 1.2 kb and the other having a size of about 0.7 kb. This DNA fragment includes the murC gene derived from coryneform bacteria.

First Hit

L3: Entry 10 of 17 File: EPAB Jan 7, 1999

DOCUMENT-IDENTIFIER: EP 889123 A2
TITLE: MurC gene of Staphylococcus aureus coding for UDP-N-acetylmuramate:L-alanine Ligase

Abstract Text (1):
CHG DATE=19990905 STATUS=O> The invention provides MurC polypeptides and polynucleotides encoding MurC polypeptides and methods for producing such polypeptides by recombinant techniques. Also provided are methods for utilizing MurC polypeptides to screen for antibacterial compounds.

First Hit

L3: Entry 11 of 17 File: DWPI Apr 3, 2003

DERWENT-ACC-NO: 2003-441048
DERWENT-WEEK: 200341

TITLE: Novel crystallized recombinant polypeptides from Staphylococcus aureus, Streptococcus pneumoniae and Helicobacter pylori and which are involved in membrane biosynthesis, useful as targets for pathogenic bacteria

INVENTOR: ALAM, M Z; AWREY, D ; BEATTIE, B ; CANADIEN, V ; DHARAMSI, A ; DOMAGALA, M ; EDWARDS, A ; HOUSTON, S ; KANAGARAJAH, D ; LI, Q ; MANSOURY, K ; MCDONALD, M ; NECAKOV, S ; NG, I ; PINDER, B ; SHELDRICK, B ; VALLEE, F ; VEDADI, M ; VIOLA, C ; WREZEL, O

PATENT-ASSIGNEE: AFFINIUM PHARM INC (AFFIN)

PRIORITY-DATA: 2001US-343946P (December 27, 2001), 2001US-324449P (September 24, 2001), 2001US-324504P (September 24, 2001), 2001US-326269P (October 1, 2001), 2001US-326887P (October 3, 2001), 2001US-339560P (October 24, 2001), 2001US-337471P (October 25, 2001), 2001US-340000P (October 26, 2001), 2001US-340002P (October 26, 2001), 2001US-340027P (October 26, 2001), 2001US-341767P (December 18, 2001), 2001US-344307P (December 21, 2001)

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PATENT-FAMILY:

	PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
☐	WO 2003027139 A2	April 3, 2003	E	312	C07K014/195

DESIGNATED-STATES: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT RO RU SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG US UZ VC VN YU ZA ZM ZW AT BE BG CH CY CZ DE DK EA EE ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SK SL SZ TR TZ UG ZM ZW

APPLICATION-DATA:

PUB-NO	APPL-DATE	APPL-NO	DESCRIPTOR
WO2003027139A2	September 24, 2002	2002WO-CA01443	

INT-CL (IPC): C07 K 14/195

ABSTRACTED-PUB-NO: WO2003027139A

BASIC-ABSTRACT:

NOVELTY - A crystallized recombinant polypeptide (I) comprising amino acid sequence of polypeptides from Staphylococcus aureus, Streptococcus pneumoniae, Helicobacter pylori and Pseudomonas aeruginosa and which are involved in membrane biosynthesis, or amino acid sequences having at least 90 % identity with the polypeptide

sequence, where the polypeptide is in crystal form, is new.

DETAILED DESCRIPTION - A crystallized recombinant polypeptide (I) comprises the amino acid sequence of polypeptides (P) involved in membrane biosynthesis, which includes FtsZ cell division protein (FtsZ), (3R)-hydroxymyristol acyl carrier protein dehydratase (FabZ), acyl carrier protein synthase (AcpS), (3-oxoacyl-(acyl-carrier-protein) synthase III (FabH), teichoic acid biosynthesis protein D (TagD), and Spo0B-associated GTP-binding protein (Obg) from S. aureus 3-ketoacyl-acyl-carrier protein reductase (FabG), UDP-N-acetylmuramoylala- nine-D-glutamate ligase (MurD) and L-alanine adding enzyme (UDP-N-acetyl-muramate:alanine ligase) (MurC) from H. pylori, acyl carrier protein synthase (AcpS) from S. pneumoniae, 3-oxoacyl-(acyl-carrier-protein)reductase (FabG) from P. aeruginosa. (I) comprises an amino acid sequence having at least 90 or 95 % identity with the amino acid sequence of (P), or comprises an amino acid sequence encoded by a polynucleotide that hybridize under stringent conditions to the complementary strand of a polynucleotide having a sequence encoding any of (P). (I) is in a crystal form.

INDEPENDENT CLAIMS are also included for:

(1) a sample (II) comprising (P), labeled with a heavy atom, or enriched in nuclear magnetic resonance (NMR) isotope;

(2) a crystallized complex comprising the recombinant polypeptides as above and a co-factor or a small organic molecule, where the complex is in a crystal form;

(3) a host cell comprising a nucleic acid encoding (P), where the culture of the host cell produces 1 mg of the polypeptide/l of culture and the polypeptide is at least one-third soluble as measured by gel electrophoresis;

(4) a composition (C) comprising an isolated, recombinant polypeptide, FabG, AcpS, MurD, MurC, TagD or AcpS as above, its 95 % identical sequence or an amino acid sequence encoded by a polynucleotide that hybridizes under stringent conditions to the complementary strand of polynucleotide encoding any of the above polypeptide, where the polypeptide is at least 90 % pure in a sample of the composition;

(5) a composition (III) same as (C), but comprising only FtsZ, FabZ, Obg, FabG or FabH polypeptide;

(6) a complex comprising a polypeptide of (III) and FTSA cell division protein;

(7) a complex comprising polypeptide of (IV) one or more of 50S ribosomal protein (RP) L22, 30S RP S7, 30S RP S5, 50S RP L5, 50S RP L5, 50S RP L6, 50S RP L4, 30S RP S4, 50S RPL3, 50S RP L2, 30S RP S2, RNA polymerase alpha , RNA polymerase beta and beta ';

(8) a complex comprising FabH and leukotoxin LukM and 16 kDa unidentified protein;

(9) a complex comprising FabH and 50S RP L16 and leukotoxin LukM;

(10) a complex comprising FabG and a 25 kDa unidentified protein;

(11) an isolated, recombinant polypeptide comprising at least 90 % identity with FtsZ polypeptide, or an amino acid sequence encoded by a polynucleotide that hybridizes under stringent conditions to the complementary strand of polynucleotide encoding FtsZ, where the polypeptide includes Asp at position 155 and/or 354;

(12) an isolated, recombinant polypeptide comprising at least 90 % identity with FabZ polypeptide from S. aureus, or an amino acid sequence encoded by a polynucleotide that hybridizes under stringent conditions to the complementary strand of polynucleotide encoding FabZ, where the polypeptide includes Leu at position 15 and Asn at position 113;

(13) an isolated recombinant polypeptide, comprising at least 90 % identity with FabG from H. pylori or an amino acid sequence encoded by a polynucleotide that hybridizes under stringent conditions to the complementary strand of a polynucleotide having a sequence encoding FabG, where the polypeptide includes Cys at position 55;

(14) an isolated, recombinant polypeptide comprising at least 90 % identity with AcpS, where the polypeptide includes one or more of the amino acid residues at the specified position of the polypeptide: Lys at position 11;

(15) an isolated, recombinant polypeptide, comprising at least 90 % identity with MurD or an amino acid sequence encoded by a polynucleotide that hybridizes under stringent conditions to the complementary strand of polynucleotide encoding MurD, where the polypeptide includes Ala at position 16;

(16) an isolated, recombinant polypeptide comprising at least 90 % identity with MurC polypeptide, or an amino acid sequence encoded by a polynucleotide that hybridizes under stringent conditions to the complementary strand of polynucleotide encoding MurC, where the polypeptide includes Thr at position 385, Ile at position 427 and Lys at position 429;

(17) an isolated, recombinant polypeptide comprising at least 95 % identity with Obg polypeptide, or an amino acid sequence encoded by a polynucleotide that hybridizes under stringent conditions to the complementary strand of polynucleotide encoding Obg, where the polypeptide includes Phe at position 339; and

(18) an isolated, recombinant polypeptide comprising at least 90 % identity with FabG polypeptide, or an amino acid sequence encoded by a polynucleotide that hybridizes under stringent conditions to the complementary strand of polynucleotide encoding FabG, where the polypeptide includes Ala at position 58.

ACTIVITY - Antibacterial.

No biological data is given.

MECHANISM OF ACTION - Vaccine.

USE - (I) is useful for designing a modulator for the prevention or treatment of S. aureus, H. pylori, S. pneumoniae, and P. aeruginosa-related disease or disorder. (I) is also useful for identifying small molecules that bind to a recombinant polypeptide. (All claimed.) The structural and functional information of (I) aid in the discovery and design of therapeutic and diagnostic molecules. The crystal structure is useful to make a structural or computer model of the polypeptide, complex or its portion. (I) is also useful for determining crystal structure of a homolog of (P). A protein complex comprising (P) is useful for identifying modulators of the protein complex. Detecting the presence of (P) is useful for diagnosing a patient suffering from a disease or disorder of a pathogenic species. The diagnostic assays are useful for monitoring the effectiveness of an anti-pathogenic treatment in an individual suffering from a disease or disorder of such pathogen. (I) and the recombinant polypeptides are useful for inducing an immunological response in an individual and as an antigen for vaccination of a host

to produce specific antibodies which protect against invasion of bacteria, for example by blocking adherence of bacteria to damaged tissue.

ABSTRACTED-PUB-NO: WO2003027139A
EQUIVALENT-ABSTRACTS:

CHOSEN-DRAWING: Dwg.0/99

DERWENT-CLASS: A89 B04 D16 D22 K08 S03
CPI-CODES: A12-V01; A12-V03C2; B04-C01G; B04-F10; B04-F10A6; B04-F10B3; B04-F10B4; B04-L03D0E; B04-L0600E; B04-L0800E; B04-N03A0E; B05-A01B; B05-A03A; B05-A03B; B05-A04; B05-B01D; B05-B02A3; B05-C03; B05-C06; B05-C07; B05-C08; B10-C04C; B11-C07B; B11-C08A; B11-C08G1; B12-K04A4; B12-K04E; B14-A01; B14-A01A6; B14-A01B2; B14-A01B4; B14-S11B; D05-C03B; D05-C03E; D05-C03G; D05-C12; D05-H14; D05-H17A3; D05-H17A6; D09-D09-A01C; K08-E; K09-E;
EPI-CODES: S03-E07C; S03-E14H4;

First Hit

L3: Entry 12 of 17 File: DWPI Mar 27, 2003

DERWENT-ACC-NO: 2003-468119
DERWENT-WEEK: 200344

TITLE: Novel crystallized recombinant polypeptides from Staphylococcus aureus, Streptococcus pneumoniae and Escherichia coli and which are involved in membrane biosynthesis, useful as targets for pathogenic bacteria

Basic Abstract Text (4):
(2) a crystallized complex comprising the recombinant polypeptides, MurD, MurC, MvaK1, AccA, FemD, DdlA, and GlmM as above and a co-factor or a small organic molecule, where the complex is in a crystal form;

Basic Abstract Text (8):
(6) a composition (III) comprising MurC polypeptide, a polypeptide having at least 90% identity with the aa sequence of the polypeptide, or a polypeptide encoded by a polynucleotide that hybridizes under stringent conditions to the complementary strand of a polynucleotide that encodes MurC polypeptide, where the polypeptide is at least 90% pure in a sample of the composition;

Basic Abstract Text (10):
(8) a host cell comprising a nucleic acid encoding a MurC, MurB, MvaK1, FemD, DdlA, GlmM, AccA or MurD polypeptide from S. pneumoniae, as in (I), where the culture of the host cell produces 1 mg of the polypeptide/l of culture and the polypeptide is at least one-third soluble as measured by gel electrophoresis;

First Hit

L3: Entry 12 of 17 File: DWPI Mar 27, 2003

DERWENT-ACC-NO: 2003-468119
DERWENT-WEEK: 200344

TITLE: Novel crystallized recombinant polypeptides from Staphylococcus aureus, Streptococcus pneumoniae and Escherichia coli and which are involved in membrane biosynthesis, useful as targets for pathogenic bacteria

INVENTOR: ALAM, M Z; AWREY, D ; BEATTIE, B ; DHARAMSI, A ; DOMAGALA, M ; EDWARDS, A ; HOUSTON, S ; KANAGARAJAH, D ; MANSOURY, K ; MCDONALD, M ; NETHERY, K ; NG, I ; PINDER, B ; VEDADI, M ; VIOLA, C ; WREZEL, O

PATENT-ASSIGNEE: AFFINIUM PHARM INC (AFFIN)

PRIORITY-DATA: 2001US-341949P (December 19, 2001), 2001US-323992P (September 21, 2001), 2001US-324152P (September 21, 2001), 2001US-324692P (September 25, 2001), 2001US-339924P (October 26, 2001), 2001US-350973P (October 29, 2001), 2001US-340924P 340924P (October 30, 2001), 2001US-333666P (November 27, 2001), 2001US-341732P (December 18, 2001), 2001US-341776P (December 18, 2001)

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PATENT-FAMILY:

	PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
☐	WO 2003025007 A2	March 27, 2003	E	325	C07K014/195

DESIGNATED-STATES: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT RO RU SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG US UZ VC VN YU ZA ZM ZW AT BE BG CH CY CZ DE DK EA EE ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SK SL SZ TR TZ UG ZM ZW

APPLICATION-DATA:

PUB-NO	APPL-DATE	APPL-NO	DESCRIPTOR
WO2003025007A2	September 20, 2002	2002WO-CA01428	

INT-CL (IPC): C07 K 14/195

ABSTRACTED-PUB-NO: WO2003025007A
BASIC-ABSTRACT:

NOVELTY - A crystallized recombinant polypeptide (I) comprising the amino acid (aa) sequence of polypeptides from Staphylococcus aureus, Streptococcus pneumoniae and Escherichia coli and which are involved in membrane biosynthesis, or aa sequences having at least 90% identity with the polypeptide sequence, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for:

(1) a sample (II) comprising isolated, recombinant polypeptide as in (I), labeled with a heavy atom, or enriched in NMR isotope;

(2) a crystallized complex comprising the recombinant polypeptides, MurD, MurC, MvaK1, AccA, FemD, DdlA, and GlmM as above and a co-factor or a small organic molecule, where the complex is in a crystal form;

(3) an isolated, recombinant polypeptide comprising an aa sequence having at least 80% identity with GlmM polypeptide, or an aa sequence encoded by a polynucleotide that hybridizes under stringent conditions to the complementary strand of polynucleotide encoding GlmM, where the polypeptide includes Ser at position 82;

(4) an isolated, recombinant polypeptide comprising an aa sequence having at least 80% identity with MurD polypeptide from S. pneumoniae, or an aa sequence encoded by a polynucleotide that hybridizes under stringent conditions to the complementary strand of polynucleotide encoding MurD, where the polypeptide includes Gly at position 294;

(5) an isolated recombinant polypeptide, comprising an aa sequence having at least 80% identity with the aa sequence of MurD from S. aureus or an aa sequence encoded by a polynucleotide that hybridizes under stringent conditions to the complementary strand of a polynucleotide having a sequence encoding MurD, where the polypeptide includes Ile at position 17 and/or Val at position 376;

(6) a composition (III) comprising MurC polypeptide, a polypeptide having at least 90% identity with the aa sequence of the polypeptide, or a polypeptide encoded by a polynucleotide that hybridizes under stringent conditions to the complementary strand of a polynucleotide that encodes MurC polypeptide, where the polypeptide is at least 90% pure in a sample of the composition;

(7) a complex comprising the polypeptide of (III), and one or more of aerobic glycerol-3-phosphate dehydrogenase and glutamate racemase;

(8) a host cell comprising a nucleic acid encoding a MurC, MurB, MvaK1, FemD, DdlA, GlmM, AccA or MurD polypeptide from S. pneumoniae, as in (I), where the culture of the host cell produces 1 mg of the polypeptide/l of culture and the polypeptide is at least one-third soluble as measured by gel electrophoresis;

(9) an isolated, recombinant polypeptide comprising an aa sequence having at least 90% identity with the aa sequence of MurB from S. aureus, where the polypeptide includes one or more of the aa residues at the specified position of the polypeptide: Arg at position 89 or 115, and Pro at position 89;

(10) a solution comprising a recombinant polypeptide comprising aa sequence of MurB polypeptide, it's 95% identical sequence, or aa sequence encoded by a polynucleotide that hybridizes under stringent conditions to the complementary strand of polynucleotide encoding MurB, where the polypeptide has a concentration of at least 7 mg/ml in the solution;

(11) a composition (IV) comprising an isolated, recombinant polypeptide, MvaK1 as in (I), where the polypeptide is at least 90% pure in a sample of the composition;

(12) a complex comprising a polypeptide in (IV) and approximately 70 kDa unidentified protein;

(13) a composition comprising an isolated, recombinant polypeptide, AccA from E. coli, FemD, DdlA, GlmM or MurD from S. pneumoniae as in (I), where the polypeptide

is at least 90% pure in a sample of the composition;

(14) a composition (V) comprising an isolated, recombinant polypeptide, AccA from S. S. aureus, its 95% identical sequence or an aa sequence encoded by a polynucleotide that hybridizes under stringent conditions to the complementary strand of polynucleotide encoding AccA, where the polypeptide is at least 90% pure in a sample sample of the composition;

(15) a complex comprising a polypeptide in (V) and malate:quinone oxidoreductase;

(16) an isolated, recombinant polypeptide, comprising a sequence having at least 90% 90% identity with the aa sequence of AccA from S. aureus or an aa sequence encoded by a polynucleotide that hybridizes under stringent conditions to the complementary strand of polynucleotide encoding AccA, where the polypeptide includes Val at position 93; and

(17) an isolated, recombinant polypeptide comprising an aa sequence having at least 95% identity with DdlA polypeptide, or an aa sequence encoded by a polynucleotide that hybridizes under stringent conditions to the complementary strand of polynucleotide encoding DdlA, where the polypeptide includes Asn at position 33, Ile Ile at position 170, Lys at position 250, Thr at position 257 and Glu at position 327.

ACTIVITY - Antibacterial.

No biological data given.

MECHANISM OF ACTION - Vaccine; Modulator of (P).

USE - (I) is useful for designing a modulator for the prevention or treatment of S. aureus, E. coli or S. pneumoniae related disease or disorder. (I) is also useful for for identifying small molecules that bind to a polypeptide (claimed).

The structural and functional information of (I) aid in the discovery and design of therapeutic and diagnostic molecules. The crystal structure is useful to make a structural or computer model of the polypeptide, complex or its portion. (I) is also also useful for determining crystal structure of a homolog of (P). A protein complex complex comprising (P) is useful for identifying modulators of the protein complex. Detecting the presence of (P) is useful for diagnosing a patient suffering from a disease or disorder of a pathogenic species. The diagnostic assays are useful for monitoring the effectiveness of an anti-pathogenic treatment in an individual suffering from a disease or disorder of such pathogen. (I) and the recombinant polypeptides are useful for inducing an immunological response in an individual and as an antigen for vaccination of a host to produce specific antibodies which protect protect against invasion of bacteria, for e.g. by blocking adherence of bacteria to damaged tissue. The polypeptides are also useful for developing antimicrobial agents agents which are useful as surface disinfectants, topical pharmaceuticals, personal hygiene applications, additive to cell culture medium and systemic pharmaceutical products, and as food preservative or in treating food products to eliminate potential pathogens.

ABSTRACTED-PUB-NO: WO2003025007A
EQUIVALENT-ABSTRACTS:

CHOSEN-DRAWING: Dwg.0/108

DERWENT-CLASS: A96 B04 D16 K08

CPI-CODES: A12-V01; B04-C01G; B04-E02; B04-F0100E; B04-L01; B04-N03A0E; B05-A01B; B05-A02; B05-A03A; B05-A03B; B05-A04; B05-B02C; B05-C03; B05-C06; B05-C07; B05-C08; B10-A07; B10-C04E; B10-E04C; B10-E04D; B11-C08E; B11-C08F; B11-C10; B12-K04E; B14-A01; D05-A01A4; D05-A01B; D05-H08; D05-H09; D05-H12A; D05-H14; D05-H17A6; K08-X; K09-B;

First Hit

L3: Entry 13 of 17 File: DWPI Feb 7, 2002

DERWENT-ACC-NO: 2002-360841
DERWENT-WEEK: 200239

TITLE: Analyzing a sample for measuring enzyme activity and assaying biochemical processes, by direct adsorption scintillation assay

INVENTOR: CHEN, Z; YUAN, Z

PATENT-ASSIGNEE: CHEN Z (CHENI), YUAN Z (YUANI)

PRIORITY-DATA: 1998US-074272P (February 10, 1998), 1999US-0248158 (February 9, 1999) 1999)

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PATENT-FAMILY:

	PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
☐	US 20020015678 A1	February 7, 2002		015	A61K051/00

APPLICATION-DATA:

PUB-NO	APPL-DATE	APPL-NO	DESCRIPTOR
US20020015678A1	February 10, 1998	1998US-074272P	Provisional
US20020015678A1	February 9, 1999	1999US-0248158	

INT-CL (IPC): A61 K 51/00; C12 Q 1/00; G01 N 33/53; G01 N 33/542; G01 N 33/543; G01 N 33/566; G01 N 33/567

ABSTRACTED-PUB-NO: US20020015678A
BASIC-ABSTRACT:

NOVELTY - Analyzing (M1) a sample by providing a sample containing one or more molecular species (MS), where at least one of MS is capable of stimulating scintillation, providing a scintillating material (I), where the surface of (I) adsorbs at least one of MS via general molecular property-based binding interaction and gets stimulated, and measuring the scintillation emitted by (I), is new.

DETAILED DESCRIPTION - Analyzing (M1) a sample, comprising:

(a) providing a sample containing one or more MS, where at least one of MS is capable of stimulating scintillation;

(b) providing (I), where the surface of (I) adsorbs at least one of MS via general molecular property-based binding interaction between MS and (I), and where (I) can be stimulated to scintillate by at least one of the adsorbed MS, but is generally not stimulated to scintillate by any MS which is not adsorbed; and

(c) measuring the scintillation emitted by (I).

An INDEPENDENT CLAIM is also included for a plate (II) suitable for a direct adsorption binding assay, comprised (I) and having one or more wells, or wells coated with (I).

USE - M1 is useful for analyzing a sample, which is used to identify compounds which which inhibit an enzyme catalyzed reaction from kinase, lipase, phosphotase, protease, and tRNA transferase catalyzed reactions, or from the reaction cascade or any its portion for the sequential synthesis of uridinediphosphate-N-acetylmuramic acid pentapeptide catalyzed by the enzymes MurA, MurB, MurC, MurD, MurE and MurF (claimed), and used for studying enzymatic reactions. The assays are used for analyzing samples, particularly biological and biochemical samples such as reaction substrates or products.

ADVANTAGE - These assays are convenient and inexpensive, rapid, inexpensive and convenient quantitation of a desired analyte, avoiding the need for separation of reactants and products and obviating the need for preparation of specific antibodies or receptors or the preparation of reagents derivatized with specific binding partners. It facilitates assays, and can be used for high-throughput screening of chemical libraries. Unlike prior art methods, this system need not be prepared for each step, and such a system is readily adaptable to use in high throughput screening.

ABSTRACTED-PUB-NO: US20020015678A
EQUIVALENT-ABSTRACTS:

CHOSEN-DRAWING: Dwg.0/5

DERWENT-CLASS: A96 B04 D16 S03
CPI-CODES: A12-L04; A12-W11L; B04-C03; B10-J02; B11-C07B3; B11-C08E3; B11-C10A; B12-K04; D05-A02; D05-H09;
EPI-CODES: S03-E14H; S03-E14H5;

First Hit

L3: Entry 13 of 17 File: DWPI Feb 7, 2002

DERWENT-ACC-NO: 2002-360841
DERWENT-WEEK: 200239

TITLE: Analyzing a sample for measuring enzyme activity and assaying biochemical processes, by direct adsorption scintillation assay

Basic Abstract Text (7):
USE - M1 is useful for analyzing a sample, which is used to identify compounds which inhibit an enzyme catalyzed reaction from kinase, lipase, phosphotase, protease, and tRNA transferase catalyzed reactions, or from the reaction cascade or any its portion for the sequential synthesis of uridinediphosphate-N-acetylmuramic acid pentapeptide catalyzed by the enzymes MurA, MurB, MurC, MurD, MurE and MurF (claimed), and used for studying enzymatic reactions. The assays are used for analyzing samples, particularly biological and biochemical samples such as reaction substrates or products.

SRNT

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
12 July 2001 (12.07.2001)

PCT

(10) International Publication Number
WO 01/49775 A2

(51) International Patent Classification⁷: C08G 77/00

(21) International Application Number: PCT/US01/00222

(22) International Filing Date: 4 January 2001 (04.01.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/174,484 4 January 2000 (04.01.2000) US

(71) Applicant: AVI BIOPHARMA, INC. [US/US]; Suite 200, 4575 S.W. Research Way, Corvallis, OR 97333 (US).

(72) Inventor: IVERSEN, Patrick, L.; 5902 N.W. Fair Oaks Place, Corvallis, OR 97330 (US).

(74) Agents: GORTHEY, LeeAnn et al.; Iola Pi Law Group, P.O. Box 60850, Palo Alto, CA 94306-0850 (US).

(81) Designated States (*national*): AU, CA, JP, KR.

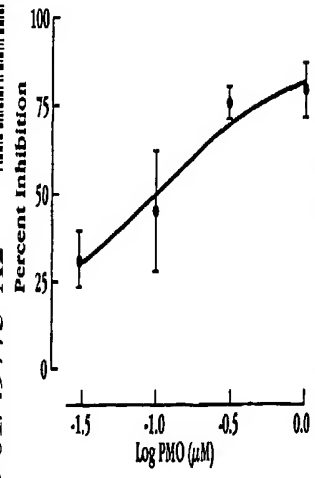
(84) Designated States (*regional*): European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR).

Published:
— *Without international search report and to be republished upon receipt of that report.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/49775 A2

(54) Title: ANTISENSE ANTIBACTERIAL CELL DIVISION COMPOSITION AND METHOD



(57) Abstract: Antisense oligomers directed to bacterial cell division and cell cycle-encoding nucleic acids are capable of selectively modulating the biological activity thereof, and are useful in treatment and prevention of bacterial infection. The antisense oligomers are substantially uncharged, and contain from 8 to 40 nucleotide subunits, including a targeting nucleic acid sequence at least 10 nucleobases in length which is effective to hybridize to (i) a bacterial tRNA or (ii) a target sequence, containing a translational start codon, within a bacterial nucleic acid which encodes a protein associated with cell division or the cell cycle. Such proteins include zipA, *sulA*, *recA*, *dicA*, *dicB*, *dicC*, *dicF*, *ftsA*, *ftsI*, *ftsN*, *ftsK*, *ftsL*, *ftsQ*, *ftsW*, *ftsZ*, *murC*, *murD*, *murE*, *murF*, *murG*, *murC*, *mraD*, *mraE*, *mraY*, *mraW*, *mraZ*, *rep*A, *ddl*B, carbamate kinase, D-ala D-ala ligase, topoisomerase, alkyl hydroperoxide reductase, thioredoxin reductase, dihydrofolate reductase, and cell wall enzyme.

First Hit

L3: Entry 14 of 17 File: DWPI Jun 17, 2003

DERWENT-ACC-NO: 2001-441859
DERWENT-WEEK: 200349

TITLE: Antisense compounds against bacterial cell division and cell cycle-encoding nucleic acids, useful for treating bacterial infections and for producing attenuated attenuated vaccines for the treatment of bacterial infections

Basic Abstract Text (2):
DETAILED DESCRIPTION - An antibacterial compound (I) comprising an uncharged antisense oligomer of 8-40 nucleotide units and which includes a targeting sequence at least 10 nucleotides in length which hybridizes to a bacterial tRNA sequence or a target sequence comprising a translational start codon within a bacterial nucleic acid which encodes a protein associated with cell division or the cell cycle. The protein is zipA, sulA, secA, dicA, dicB, dicC, dicF ftsA, ftsI, ftsK, ftsL ftsQ, ftsWftsZ, murC, murD, murE, murF, murG, minC, minD, minE, mraY, mraW, mraZ, seqA and/or ddlB proteins, carbamate kinase, D-ala D-ala ligase, topoisomerase, alkyl hydroperoxide reductase, thioredoxin reductase, dihydrofolate reductase and/or cell wall enzymes. each subunit comprises a 5- or 6- membered ring supporting a base-pairing group which can bind by Watson-Crick base pairing to a respective nucleotide in a bacterial nucleic acid sequence. Adjacent subunits are joined by uncharged linkages selected from uncharged phosphoroamidate, phosphorodiamidate, carbonate, carbamate, amide, phosphotriester, alkyl phosphonate, siloxane, sulfone, sulfonamide, sulfamate, thioformacetyl and methylene-N-methylhydroxylamin- o, or by charged linkages selected from phosphate, charged phosphoroamidate and phosphorothioate. The ratio of uncharged linkages to charged linkages in the oligomer is at least 4:1.

First Hit

L3: Entry 15 of 17 File: DWPI Mar 22, 2001

DERWENT-ACC-NO: 2001-281522
DERWENT-WEEK: 200129

TITLE: New Pseudomonas aeruginosa MurC enzyme involved in bacterial cell wall biosynthesis, useful for identifying inhibitors of enzyme which are active against both gram positive and gram negative bacteria

Basic Abstract Text (1):
NOVELTY - Purified and isolated Pseudomonas aeruginosa UDP-N-acetylmuramyl:L-alanine ligase (MurC) protein or a polypeptide (I) which has the fully defined 487 amino acid sequence (S2) given in the specification, or that is a naturally occurring mutant or polymorphic form of (S2), is new.

Basic Abstract Text (15):
(3) providing a sample that includes a MurC polypeptide such as a polypeptide having having a sequence of (S2), a polypeptide that is a functional derivative of (S2) or a polypeptide that is a naturally occurring mutant or polymorphic form of (S2);

Basic Abstract Text (16):
(4) contacting the sample with the candidate to permit the interaction of the candidate with the MurC polypeptide; and

Basic Abstract Text (23):
(II) are useful in the expression and production of P.aeruginosa MurC protein. Partial or full length (II) can be used as probes for detecting the presence of P.aeruginosa MurC DNA or RNA, and to study the effects of modulators of MurC transcription.

Basic Abstract Text (24):
Polynucleotides having sequences that are unique or specific for P.aeruginosa MurC can be used as primers in amplification reaction assays for use in tissue typing. The primers can also be used to obtain amplified P.aeruginosa MurC cDNA using MurC cDNA of the cells as an initial template. The polynucleotides can also be used in identification of various polymorphic P.aeruginosa MurC genes or the detection of an organism having a P.aeruginosa murC gene.

SRNT

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
22 March 2001 (22.03.2001)

PCT

(10) International Publication Number
WO 01/19979 A1

(51) International Patent Classification⁷: C12N 15/09, A61K 31/70

(21) International Application Number: PCT/US00/24845

(22) International Filing Date:
11 September 2000 (11.09.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/154,073 14 September 1999 (14.09.1999) US

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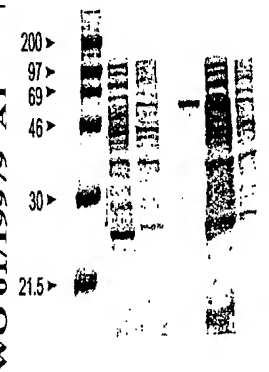
(81) Designated States (*national*): CA, JP, US.

(84) Designated States (*regional*): European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published:
– *With international search report.*
– *Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: MURC GENE AND ENZYME OF *PSEUDOMONAS AERUGINOSA*



(57) Abstract: This invention provides isolated polynucleotides that encode the MurC protein of *Pseudomonas aeruginosa*. Purified and isolated MurC recombinant proteins are also provided. Nucleic acid sequences which encode functionally active MurC proteins are described. Assays for the identification of modulators of the expression of *murC* and inhibitors of the activity of MurC, are also provided.

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First Hit

L3: Entry 16 of 17 File: DWPI Jul 27, 1999

DERWENT-ACC-NO: 1999-496654
DERWENT-WEEK: 199942

TITLE: New murC gene - used to develop suicide vehicle

Basic Abstract Text (2):
ADVANTAGE - A suicide vehicle can be developed by analyzing cell wall synthetic mechanisms using murC protein, murC gene and a DNA fragment containing the gene.

SRNT

(19)日本国特許庁（ＪＰ）　(12)**公開特許公報**（Ａ）　(11)特許出願公開番号

特開平11－196876

(43)公開日　平成11年(1999)７月27日

(51)Int.Cl.[6]	識別記号	ＦＩ	
Ｃ１２Ｎ　15/09	ＺＮＡ	Ｃ１２Ｎ　15/00	ＺＮＡＡ
9/00		9/00	
//（Ｃ１２Ｎ　15/09	ＺＮＡ		
Ｃ１２Ｒ　1:13）			

審査請求　未請求　請求項の数７　ＯＬ　（全９頁）

(21)出願番号　特願平10－6840

(22)出願日　平成10年(1998)１月16日

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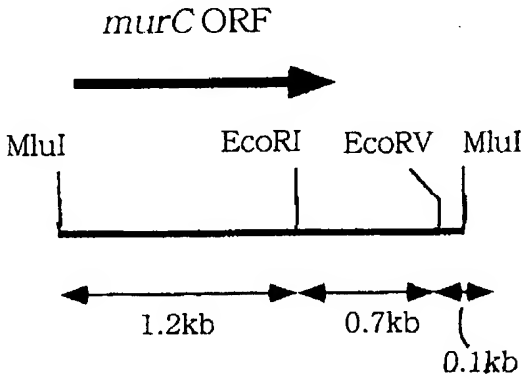
(54)【発明の名称】　新規ｍｕｒＣ遺伝子

(57)【要約】
【課題】　コリネ型細菌由来のＵＤＰ－Ｎ－アセチルムラミン酸－アラニンリガーゼ遺伝子（ｍｕｒＣ遺伝子）を提供する。
【解決手段】　コリネ型細菌の染色体ＤＮＡを制限酵素ＭｌｕＩ及びＥｃｏＲＶで処理して得られる、片端がＭｌｕＩサイト、もう一端がＥｃｏＲＶサイトであり、ＥｃｏＲＩサイトにより大きさが約１．２ｋｂ及び約０．７ｋｂの断片に分割される大きさが約１．９ｋｂのＤＮＡ断片を少なくとも含むＤＮＡ断片。本ＤＮＡ断片にコリネ型細菌由来のｍｕｒＣ遺伝子が含まれる。



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【特許請求の範囲】

【請求項1】　コリネ型細菌の染色体ＤＮＡを制限酵素ＭｌｕＩ及びＥｃｏＲＶで処理して得られる、片端がＭｌｕＩサイト、もう一端がＥｃｏＲＶサイトであり、ＥｃｏＲＩサイトにより大きさが約１．２ｋｂ及び約０．７ｋｂの断片に分割される大きさが約１．９ｋｂのＤＮＡ断片を少なくとも含むＤＮＡ断片。

【請求項2】　コリネ型細菌がブレビバクテリウム・フラバムＭＪ－２３３である、請求項1記載の断片。

【請求項3】　請求項1または2記載のＤＮＡ断片によってコードされるＵＤＰ－Ｎ－アセチルムラミン酸－アラニン　リガーゼ。

【請求項4】　配列番号2記載のアミノ酸配列、または、配列番号2記載のアミノ酸配列において1若しくは数個のアミノ酸が欠失、置換、若しくは付加されたアミノ酸配列からなるＵＤＰ－Ｎ－アセチルムラミン酸－アラニン　リガーゼ。

【請求項5】　請求項1または2記載のＤＮＡ断片に含まれるＵＤＰ－Ｎ－アセチルムラミン酸－アラニン　リガーゼをコードする遺伝子。

【請求項6】　請求項4記載のＵＤＰ－Ｎ－アセチルムラミン酸－アラニンリガーゼをコードする遺伝子。

【請求項7】　配列番号1記載の塩基配列からなるＤＮＡ、または、配列番号1記載の塩基配列からなるＤＮＡとストリンジェントな条件でハイブリダイズし、かつＵＤＰ－Ｎ－アセチルムラミン酸－アラニン　リガーゼ活性を有するタンパク質をコードするＤＮＡからなる遺伝子。

【発明の詳細な説明】

【０００１】

【発明の属する技術分野】本発明は、新規ＵＤＰ－Ｎ－アセチルムラミン酸－アラニン　リガーゼ（以下、ｍｕｒＣタンパク質と略記することがある）、それをコードする遺伝子（以下、ｍｕｒＣ遺伝子と略記することがある）、及び該遺伝子を含むＤＮＡ断片（以下、ｍｕｒＣ断片と略記することがある）に関する。

【０００２】

【従来の技術】ＵＤＰ－Ｎ－アセチルムラミン酸－アラニン　リガーゼ［ＥＣ６．３．２．８］は、微生物の細胞壁の生合成系の酵素の1つであり、ＵＤＰ－Ｎ－アセチルムラミン酸とＬ－アラニンとからＵＤＰ－Ｎ－アセチルムラモイル－アラニンを合成する反応を触媒している酵素である。

【０００３】ｍｕｒＣ遺伝子については、原核生物では大腸菌（Escherichia coli）由来の遺伝子［Nucleic Acids Res., 18, p4014, (1990)］、枯草菌（Bacillus subtilis）由来の遺伝子［Swiss Prot Accession No. P40778］、インフルエンザ菌(Haemophilus influenzae)由来の遺伝子［Science, 269, p496-512, (1995)］が単離され、その塩基配列が決定されている。

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【０００４】しかしながら、産業上重要なコリネ型細菌についてはｍｕｒＣ遺伝子に関しての研究がなされておらず、報告例がない。一般に、遺伝子はその由来によって、宿主が異なれば発現の程度が異なるため、産業上有用なコリネ型細菌でのｍｕｒＣ遺伝子の単離が望まれている。

【０００５】また、大腸菌における該酵素変異株（murCts, ST222）では、正常な細胞壁を合成することができず、４２℃の高温において溶菌してしまうことが知られている［J. Bacteriol., 112, p950-958, (1972)］。溶菌のしやすさは菌株により異なるため、細胞壁合成のメカニズムを解析し、より優れた菌体の開発のためにも、様々な性質を有する由来の異なるｍｕｒＣ遺伝子の単離も望まれている。

【０００６】

【発明が解決しようとする課題】本発明は、コリネ型細菌内で発現可能な、新規ｍｕｒＣタンパク質、それをコードする遺伝子、及び該遺伝子を含むＤＮＡ断片の提供を目的としてなされたものである。

【０００７】

【課題を解決するための手段】本発明者らは、上記課題を解決すべく鋭意研究を重ねた結果、遺伝子組み換えの手法を駆使することにより、コリネ型細菌からｍｕｒＣタンパク質及びｍｕｒＣ遺伝子が単離可能であることを見い出し、本発明を完成するに至った。

【０００８】すなわち、本発明は、コリネ型細菌の染色体ＤＮＡを制限酵素ＭｌｕＩ及びＥｃｏＲＶで処理して得られる、片端がＭｌｕＩサイト、もう一端がＥｃｏＲＶサイトであり、ＥｃｏＲＩサイトにより大きさが約１．２ｋｂ及び約０．７ｋｂの断片に分割される大きさが約１．９ｋｂのＤＮＡ断片を少なくとも含むＤＮＡ断片を提供する。好ましくは、コリネ型細菌はブレビバクテリウム・フラバムＭＪ－２３３である。

【０００９】本発明は、また、上記のＤＮＡ断片によってコードされるＵＤＰ－Ｎ－アセチルムラミン酸－アラニン　リガーゼを提供する。このようなＵＤＰ－Ｎ－アセチルムラミン酸－アラニン　リガーゼとして、具体的には、配列番号2記載のアミノ酸配列、または、配列番号2記載のアミノ酸配列において1若しくは数個のアミノ酸が欠失、置換、若しくは付加されたアミノ酸配列からなるＵＤＰ－Ｎ－アセチルムラミン酸－アラニン　リガーゼが挙げられる。

【００１０】本発明は、さらにまた、上記のＵＤＰ－Ｎ－アセチルムラミン酸－アラニンリガーゼをコードする遺伝子を提供する。このような遺伝子として、具体的には、配列番号1記載の塩基配列からなるＤＮＡ、または、配列番号1記載の塩基配列からなるＤＮＡとストリンジェントな条件でハイブリダイズし、かつＵＤＰ－Ｎ－アセチルムラミン酸－アラニン　リガーゼ活性を有するタンパク質をコードするＤＮＡからなる遺伝子が挙げ

られる。

【0011】

【発明の実施の形態】本発明のｍｕｒＣ断片は、通常は、コリネ型細菌、例えばブレビバクテリウム・フラバム（Brevibacterium flavum）ＭＪ－２３３（ＦＥＲＭ　ＢＰ－１４９７）株等の染色体ＤＮＡをＭｌｕＩ、ＥｃｏＲＶ等の制限酵素を用いて切断し、得られた断片の中から、制限酵素サイトと大きさに基づいて、あるいは、ＵＤＰ－Ｎ－アセチルムラミン酸－アラニン　リガーゼ活性に関する形質転換能に基づいて目的断片を選択することにより得られる。

【0012】具体的には、上記コリネ型細菌、例えばブレビバクテリウム・フラバムＭＪ－２３３を常法［例えば、特開昭５１－１３０５９２号公報参照］に従い培養し、培養物から菌体を集め、該菌体から染色体ＤＮＡを抽出する。染色体ＤＮＡは、例えば、特開平５－１５３７８号公報の実施例１（Ａ）に記載の方法等により菌体から容易に抽出することができる。

【0013】この染色体ＤＮＡ断片を適当な制限酵素、例えばＭｌｕＩ及びＥｃｏＲＶにより完全分解した後、適当なクローニングベクター、例えばｐＭＷ１１８（宝酒造製）に連結しプラスミドバンクを作製する。

【0014】得られたプラスミドバンクを用いて、大腸菌ｍｕｒＣ変異株（ＳＴ２２２）［J. Bacteriol., 112, p950-958, (1972)］を形質転換し、４２℃で生育可能な形質転換体を得る。この形質転換株を適当な抗生物質選択下で培養し、培養物から菌体を回収し、菌体から常法、例えばアルカリ－ＳＤＳ法によりプラスミドを抽出し、抽出したプラスミドを適当な制限酵素により分解することにより、本発明のｍｕｒＣ断片を取得することができる。

【0015】本発明のｍｕｒＣ遺伝子は、本発明においてその塩基配列の一つが配列番号１で示されるように決定されたので、それに基づいて、”オリゴ１０００Ｍ”ＤＮＡ合成装置（ベックマン社製）等の市販のＤＮＡ合成装置を用いて合成することも可能であるが、コリネ型細菌、例えばブレビバクテリウム・フラバム（Brevibacterium flavum）ＭＪ－２３３（ＦＥＲＭ　ＢＰ－１４９７）株等の染色体ＤＮＡから上記に示した方法で得られるｍｕｒＣ断片から、以下に述べる方法でも取得することができる。

【0016】まず、上記で得られるｍｕｒＣ断片をＳａｕ３ＡＩのような適当な制限酵素を用いて切り出し、得られたＤＮＡ断片を適当なクローニングベクター、例えば、ｐＵＣ１１８（宝酒造製）へサブクローニングし、エシェリヒア・コリＪＭ１０９株（宝酒造製）を形質転換する。この形質転換株を適当な抗生物質選択下で培養し、培養物から菌体を回収する。該菌体から、アルカリ－ＳＤＳ法のような公知の方法によりプラスミドを抽出し、このプラスミドに導入されたＤＮＡ断片の塩基配列

を決定することにより、本発明のｍｕｒＣ遺伝子を含むＤＮＡ断片の塩基配列が決定される。このＤＮＡ断片の塩基配列決定法としては、例えば、ジデオキヌクレオチド酵素法［dideoxy chain termination法； Sanger F. et al.,Proc. Natl. Acad. Sci. U.S.A., 74, p.5463 (1977)］が挙げられる。このＤＮＡ断片の配列中に存在するオープンリーディングフレームから、ｍｕｒＣタンパク質のアミノ酸配列が決定される。このようにして決定されたアミノ酸配列の例としては配列番号２記載のアミノ酸配列が挙げられ、またこれをコードする遺伝子は、例えば、配列番号１記載の塩基配列中の１番目から１４５８番目までの塩基配列で示されるものである。

【0017】ｍｕｒＣ遺伝子の塩基配列には、コリネ型細菌に含まれる種の間での相違や個体間で自然突然変異などにより生じ得る相違があることが予想されるが、このような相違の範囲内であれば、配列番号１に示す塩基配列の一部の塩基が他の塩基で置換されていてもよくまたは削除されていてもよく、あるいは新たに塩基が挿入されていてもよく、さらに塩基配列の一部が転位されているものであってもよく、これらも本発明のｍｕｒＣ遺伝子に包含されるものである。また、ｍｕｒＣタンパク質のアミノ酸配列の一部は、生体内の他のタンパク質に関して知られているように、突然変異などによりタンパク質の機能を実質的に変えずに変化し得ると予想される。このような１または数個のアミノ酸の欠失、置換または付加による変化は、当業者であれば容易に判別できるものであり、従って、本発明のｍｕｒＣタンパク質は、配列番号２のアミノ酸配列に相当する配列を有するものからなるものである。すなわち、配列番号２のアミノ酸配列と同一の配列をもつものだけでなく、コリネ型細菌に含まれる種の間での相違や個体間での自然突然変異などにより生じ得る相違を有するものも包含する。

【0018】また、上述のように染色体ＤＮＡから取得したｍｕｒＣ遺伝子の塩基配列に、ｍｕｒＣタンパク質の機能を実質的に損なうことがない限り、ＰＣＲを用いた変異導入手法、ＮＴＧやヒドロキシルアミン等の変異剤を用いる等の方法で、ｍｕｒＣタンパク質のアミノ酸配列に１または数個のアミノ酸の欠失、置換、付加を導入するような改変を導入することができる。得られた改変型のｍｕｒＣタンパク質の活性の確認は、改変したｍｕｒＣ遺伝子を含むＤＮＡ断片を用いて大腸菌ｍｕｒＣ変異株（ＳＴ２２２）を形質転換し、この形質転換株が適当な抗生物質選択下で生育可能かどうか見ることで行うことができる。これらの手法により得られるタンパク質も、本発明におけるｍｕｒＣタンパク質に含まれるものである。

【0019】また、本発明において、ストリンジェントな条件でハイブリダイズする遺伝子とは、一般に、配列番号１記載の塩基配列と７０％以上、好ましくは８０％以上の相同性を有する塩基配列をいう。これらの塩基配

列について、上記手法により、ｍｕｒＣタンパク質活性の有無を確認できたものは、本発明におけるｍｕｒＣ遺伝子に含まれるものである。

【００２０】なお、ｍｕｒＣタンパク質は、常法に従い、ｍｕｒＣ遺伝子を適当な宿主で発現させ、発現させたｍｕｒＣタンパク質を、常法に従って単離することによって得ることができる。

【００２１】

【実施例】以下、実施例によりさらに具体的に説明するが、実施例は本発明の範囲を限定するものではない。

【００２２】（Ａ）　ブレビバクテリウム・フラバムＭＪ－２３３の全ＤＮＡの抽出

ブレビバクテリウム・フラバムＭＪ－２３３（ＦＥＲＭ　ＢＰ－１４９７）を、半合成培地であるＡ培地［組成：尿素　２ｇ，$(NH_4)_2SO_4$　７ｇ、K_2HPO_4　０．５ｇ，KH_2PO_4　０．５ｇ、$MgSO_4 \cdot 7H_2O$　０．５ｇ、$FeSO_4 \cdot 7H_2O$　６ｍｇ、$MnSO_4 \cdot 4 \sim 6H_2O$　６ｍｇ、酵母エキス　２．５ｇ、カザミノ酸　５ｇ、ビオチン　２００μｇ、塩酸チアミン　２００μｇ、グルコース　２０ｇを蒸留水に溶解して１リットルとする］１リットル中で対数増殖期後期まで培養した後に菌体を回収した。

【００２３】得られた菌体を、リゾチームを１０ｍｇ/ｍｌの濃度で含有する溶液［組成：１０ｍＭ　ＮａＣｌ、２０ｍＭ　トリス緩衝液（ｐＨ８．０）、１ｍＭ　ＥＤＴＡ・２Ｎａ］１５ｍｌに懸濁した。該懸濁液にプロテナーゼＫを１００μｇ/ｍｌの最終濃度で添加し、これを３７℃で１時間インキュベートした。次に、ドデシル硫酸ナトリウムを最終濃度が０．５％になるように添加し、５０℃で６時間インキュベートして溶菌させた。得られた溶菌液に等量のフェノール／クロロホルム溶液を添加して室温で１０分間穏やかに振盪した後、その全量を１０～１２℃で２０分間、５，０００×ｇの遠心分離に供し、その上清画分を分取した。該上清画分中に酢酸ナトリウムをその濃度が０．３Ｍとなるように添加し、次いで2倍量のエタノールを穏やかに添加した。水層とエタノール層の間に存在するＤＮＡをガラス棒で掬い取り、これを７０％　エタノールで洗浄して風乾した。得られたＤＮＡは、溶液［組成：１０ｍＭ　トリス緩衝液（ｐＨ７．５）、１ｍＭ　ＥＤＴＡ・２Ｎａ］５ｍｌを加えて４℃で一晩静置した後、実験に供した。

【００２４】（Ｂ）ｍｕｒＣ遺伝子を含む染色体ＤＮＡ　ＭｌｕＩ断片の取得

上記実施例（Ａ）で得られた染色体１μｇを制限酵素ＭｌｕＩを用いて完全分解した後、末端をブラントエンド処理キット（宝酒造社製）を用いて平滑末端とした。

【００２５】それぞれ最終濃度が、５０ｍＭ　トリス－塩酸緩衝液（ｐＨ７．９），１０ｍＭ　$MgCl_2$，２０ｍＭ　ジチオスレイトール，１ｍＭ　ＡＴＰ，Ｔ４ＤＮＡリガーゼ　１ｕｎｉｔ／５０μｌ，ｐＵＣ１１８ベクターＨｉｎｃＩＩ分解脱リン酸化処理物（宝酒造製）　５０ｎｇ／５０μｌ，染色体ＭｌｕＩ完全分解平滑末端処理物　２５０ｎｇ／５０μｌとなるように各成分を添加し、１６℃で３時間反応させて、染色体ＭｌｕＩ完全分解平滑末端処理物を結合させた。

【００２６】ついで、常法［J. Mol. Biol., 53, 159(1970)参照］に従って、得られた溶液を用いて大腸菌ｍｕｒＣ変異株（ＳＴ２２２）を形質転換した。得られた形質転換菌を選択培地［組成：トリプトン　１０ｇ，酵母エキス　５ｇ，ＮａＣｌ　５ｇ，寒天　１５ｇ，アンピシリン　５０ｍｇを蒸留水に溶解して１リットルとする］に塗抹し、４２℃で１６時間培養した。

【００２７】選択培地上に生育した菌株を、アンピシリンを最終濃度で５０μｇ/ｍｌ含有するＬ培養液［トリプトン　１０ｇ，酵母エキス　５ｇ，ＮａＣｌ　５ｇを蒸留水に溶解して１リットルとする］に植菌し、これを３７℃で７時間培養した。培養液を４℃で１０分間８，０００×ｇの遠心分離にかけて菌体を回収した。回収した菌体からアルカリ－ＳＤＳ法［T. Maniatis, E.F. Fritsch, J. Sambrook, Molecular cloning, p.90-91(1982)参照］によりプラスミドを抽出した。

【００２８】抽出したプラスミドはいずれも、大きさ約２．０ｋｂの断片を含んでいた。

【００２９】（Ｃ）ｍｕｒＣ遺伝子を含む染色体ＤＮＡ　ＭｌｕＩ断片のマップの作製

上記実施例（Ｂ）で得られたプラスミドＤＮＡを制限酵素ＭｌｕＩ，ＥｃｏＲＶ，ＥｃｏＲＩでそれぞれ分解した。これらについて０．８％アガロースゲルにて電気泳動を行うことにより、ｍｕｒＣ断片を含む挿入ＤＮＡ断片の大きさを決定した。

【００３０】切断断片の大きさは、大腸菌のラムダファージ（λｐｈａｇｅ）ＤＮＡを制限酵素ＨｉｎｄＩＩＩで切断して得られる分子量既知のＤＮＡ断片の同一アガロースゲル上での泳動距離で描かれる標準線に基づき、算出した。

【００３１】制限酵素ＥｃｏＲＶにより１．９ｋｂと０．１ｋｂ、ＥｃｏＲＩにより１．２ｋｂと０．８ｋｂの大きさの断片が生じた。これに基づき、マップを作製すると図１のようになった。

【００３２】（Ｄ）ｍｕｒＣ遺伝子を含む染色体ＤＮＡ領域の特定

上記実施例（Ｃ）で作製したマップをもとに、上記断片中のｍｕｒＣ遺伝子を含む領域を上記大腸菌ｍｕｒＣ変異株を用いて特定した。

【００３３】大きさが約２．０ｋｂのＭｌｕＩ断片内に存在する、ＥｃｏＲＩ、ＥｃｏＲＶサイトを利用して、種々の大きさをもつＤＮＡ断片をプラスミドｐＭＷ１１８またはｐＭＷ１１９（宝酒造製）に連結し、大腸菌ｍｕｒＣ変異株を形質転換し、４２℃における生育を指標に、ｍｕｒＣ遺伝子の活性の有無を確認した。

【００３４】その結果、大きさが約１．９ｋｂのＭｌｕＩ－ＥｃｏＲＶ断片が挿入された場合にのみ、大腸菌ｍｕｒＣ変異株の４２℃における生育を相補することができ、ｍｕｒＣ遺伝子は、大きさが約１．９ｋｂのＭｌｕＩ－ＥｃｏＲＶサイトの間に存在するものと考えられた（図２参照）。

【００３５】（Ｅ）ｍｕｒＣ遺伝子の全塩基配列の決定
大きさが約１．９ｋｂのＭｌｕＩ－ＥｃｏＲＶ断片を制限酵素ＳａｕＡＩを用いて３７℃で処理して部分分解した。また、ｐＵＣ１１８ベクター（宝酒造製）を制限酵素ＢａｍＨＩで完全に分解した。得られたベクターＤＮＡ断片と部分分解ＤＮＡ断片とを混合し、この混合液に、それぞれ最終濃度が、５０ｍＭ　トリス－塩酸緩衝液（ｐＨ７．９），１０ｍＭ　$MgCl_2$，２０ｍＭ　ジチオスレイトール，１ｍＭ　ＡＴＰ，Ｔ４ＤＮＡリガーゼ　１ｕｎｉｔ／５０μｌとなるように各成分を添加し、ベクターＤＮＡ断片と部分分解ＤＮＡ断片とを結合させた。

【００３６】同様に大きさが約１．９ｋｂのＭｌｕＩ－ＥｃｏＲＶ断片を制限酵素ＴａｃＩを用いて３７℃で処理して部分分解した。また、ｐＵＣ１１８ベクター（宝酒造製）を制限酵素ＡｃｃＩで完全に分解した。得られたベクターＤＮＡ断片と部分分解ＤＮＡ断片とを混合し、この混合液に、それぞれ最終濃度が、５０ｍＭ　トリス－塩酸緩衝液（ｐＨ７．９），１０ｍＭ　$MgCl_2$，２０ｍＭ　ジチオスレイトール，１ｍＭ　ＡＴＰ，Ｔ４ＤＮＡリガーゼ　１ｕｎｉｔ／５０μｌとなるように各成分を添加し、ベクターＤＮＡ断片と部分分解ＤＮＡ断片とを結合させた。

【００３７】ついで、常法［J. Mol. Biol., 53, 159(1970)参照］に従って、得られた溶液を各々用いて大腸菌ＪＭ１０９（宝酒造製）を形質転換した。得られた形質転換菌を選択培地［組成：トリプトン　１０ｇ，酵母エキス　５ｇ，ＮａＣｌ　５ｇ，寒天　１５ｇ，アンピシリン５０ｍｇを蒸留水に溶解して１リットルとする］に塗抹し、４２℃で１６時間培養した。

【００３８】選択培地上に生育した菌株を、アンピシリンを最終濃度で５０μｇ/ｍｌ含有するＬ培養液［トリプトン　１０ｇ，酵母エキス　５ｇ，ＮａＣｌ　５ｇを蒸留水に溶解して１リットルとする］に植菌し、これを３７℃で７時間培養した。培養液を４℃で１０分間８，０００×ｇの遠心分離にかけて菌体を回収した。回収した菌体からアルカリ－ＳＤＳ法［T. Maniatis, E.F. Fritsch, J. Sambrook, Molecular cloning, p.90-91(1982)参照］によりプラスミドを抽出した。

【００３９】抽出したプラスミドＤＮＡを用いて、ベクターｐＵＣ１１８に挿入されたＤＮＡ断片の塩基配列を決定した。ｐＵＣ１１８に挿入されたＤＮＡ断片の塩基配列の決定には、パーキン・エルマー社製のダイプライマーサイクルシーケンスキットを用いて行った。そして、これらの個々の配列の連結を、パーキン・エルマー社製のシークエンス解析ソフト　オートアッセンブラー（Ａｕｔｏａｓｓｅｍｂｌｅｒ）を用いて行った。その結果、上記１．９ｋｂのＭｌｕＩ－ＥｃｏＲＶのＤＮＡ断片には、既知の大腸菌のｍｕｒＣタンパク質と相同性（３７％）を持つタンパク質をコードする遺伝子（配列番号１に記載するオープン・リーディング・フレーム）を含有していることが判明した。即ち、得られたＤＮＡ断片にコードされるタンパク質のアミノ酸一次構造（配列番号２）と既知の大腸菌のｍｕｒＣタンパク質のアミノ酸一次構造との相同性の比較によっても、上記１．９ｋｂのＭｌｕＩ－ＥｃｏＲＶのＤＮＡ断片がブレビバクテリウム・フラバムＭＪ－２３３のｍｕｒＣ遺伝子を含んでいることが支持された。

【００４０】

【発明の効果】本発明により提供されるｍｕｒＣタンパク質、ｍｕｒＣ遺伝子および該遺伝子を含むＤＮＡ断片を用いて、細胞壁合成機構を解析することにより、人為的細胞複製制御、耐溶菌株育種、人為的溶菌による自殺ビーイクルの開発が可能となる。

【００４１】

【配列表】配列番号：１
配列の長さ：１４５８
配列の型：核酸
鎖の数：二本鎖
トポロジー：直鎖状
配列の種類：Genomic DNA
起源：
生物名：ブレビバクテリウム・フラバム（Brevibacterium flavum）
株名：ＭＪ－２３３
配列の特徴：
特徴を表す記号：ＣＤＳ
存在位置：1..1458
特徴を決定した方法：Ｅ

配列

```
GTG ACC ACT CCA CAC TTG GAT TCT GCA CAA GAT ATT GAT CTG TCC CGC     48
Val Thr Thr Pro His Leu Asp Ser Ala Gln Asp Ile Asp Leu Ser Arg
 1               5                  10                  15
GTC CAC CTC ATC GGT ATT GGC GGA GCC GGA ATG TCT GGC GTT GCC CGA     96
Val His Leu Ile Gly Ile Gly Gly Ala Gly Met Ser Gly Val Ala Arg
            20                  25                  30
```

```
     9
ATC CTG CTT GCC CGC GGT AAG ACA GTC ACT GGT TCC GAT GCC AAA GAT    144
Ile Leu Leu Ala Arg Gly Lys Thr Val Thr Gly Ser Asp Ala Lys Asp
        35                  40                  45
TCC CGC ACC TTG CTT CCA CTC CGC GCC GTG GGA GCC ACC ATC GCA GTG    192
Ser Arg Thr Leu Leu Pro Leu Arg Ala Val Gly Ala Thr Ile Ala Val
     50                  55                  60
GGA CAC GCC GCG GAA AAC CTT GAG CTT TCC GGC GAA CTT CCC ACC GTC    240
Gly His Ala Ala Glu Asn Leu Glu Leu Ser Gly Glu Leu Pro Thr Val
 65                  70                  75                  80
GTG GTG ACC TCT TTT GCC GCC ATT CCG CAA GAC AAC CCG GAA CTT GTT    288
Val Val Thr Ser Phe Ala Ala Ile Pro Gln Asp Asn Pro Glu Leu Val
                85                  90                  95
CGT GCA CGT GAA GAA GGC ATT CCG GTT ATT CGT CGC TCC GAT CTG TTG    336
Arg Ala Arg Glu Glu Gly Ile Pro Val Ile Arg Arg Ser Asp Leu Leu
            100                 105                 110
GGC GAA TTG CTG GAA GGC TCC ACC CAG GTC TTG ATC GCG GGT ACC CAC    384
Gly Glu Leu Leu Glu Gly Ser Thr Gln Val Leu Ile Ala Gly Thr His
        115                 120                 125
GGT AAG ACC TCC ACC ACC TCT ATG TCT GTG GTA GCT ATG CAG GCA GCG    432
Gly Lys Thr Ser Thr Thr Ser Met Ser Val Val Ala Met Gln Ala Ala
    130                 135                 140
GGC ATG GAT CCA AGC TTT GCT ATC GGC GGA CAG CTC AAC AAG GCT GGC    480
Gly Met Asp Pro Ser Phe Ala Ile Gly Gly Gln Leu Asn Lys Ala Gly
145                 150                 155                 160
ACC AAT GCG CAC CAT GGA ACT GGT GAG GTC TTT ATC GCT GAA GCA GAT    528
Thr Asn Ala His His Gly Thr Gly Glu Val Phe Ile Ala Glu Ala Asp
                165                 170                 175
GAA TCT GAC GCA TCG CTG CTG CGC TAC AAG CCA AAT GTT GCA GTG GTC    576
Glu Ser Asp Ala Ser Leu Leu Arg Tyr Lys Pro Asn Val Ala Val Val
            180                 185                 190
ACC AAT GTG GAA CCA GAC CAC CTG GAC TTC TTT AAA ACC CCT GAA GCC    624
Thr Asn Val Glu Pro Asp His Leu Asp Phe Phe Lys Thr Pro Glu Ala
        195                 200                 205
TAC TTC CAA GTG TTC GAC GAT TTC GCA GGA CGC ATC ACC CCG AAC GGC    672
Tyr Phe Gln Val Phe Asp Asp Phe Ala Gly Arg Ile Thr Pro Asn Gly
    210                 215                 220
AAG CTG GTT GTG TGC CTG AAC GAT CCT CAC GCA GCG GAG CTG GGG GAG    720
Lys Leu Val Val Cys Leu Asn Asp Pro His Ala Ala Glu Leu Gly Glu
225                 230                 235                 240
AGG TCT GTC CGC AAG GGT ATC AAG ACT GTT GGT TAT GGT ACC GCT GAC    768
Arg Ser Val Arg Lys Gly Ile Lys Thr Val Gly Tyr Gly Thr Ala Asp
                245                 250                 255
GCA GTA CAG GCA CAC CCT GAG GTT CCA GCG ATG GCT ACC ATC GTG GAT    816
Ala Val Gln Ala His Pro Glu Val Pro Ala Met Ala Thr Ile Val Asp
            260                 265                 270
TCC CAA GTT GTC GCA GAA GGC ACC CGC GCC ACC ATC AAC ATC GAT GGA    864
Ser Gln Val Val Ala Glu Gly Thr Arg Ala Thr Ile Asn Ile Asp Gly
        275                 280                 285
CAG GAA GTA TCT GTG ATT CTT CAA ATC CCT GGT GAT CAC ATG GTA CTC    912
Gln Glu Val Ser Val Ile Leu Gln Ile Pro Gly Asp His Met Val Leu
```

１１　　１２

290　　295　　300

AAC GGT GCA GCC GCC CTG CTG GCC GGA TAC CTG GTG GGT GGG GAC GTC　960
Asn Gly Ala Ala Ala Leu Leu Ala Gly Tyr Leu Val Gly Gly Asp Val
305　　310　　315　　320
GAC AAG CTT GTT GAA GGC TTG TCG GAT TTC TCC GGC GTG CGA CGC CGC　1008
Asp Lys Leu Val Glu Gly Leu Ser Asp Phe Ser Gly Val Arg Arg Arg
325　　330　　335
TTT GAG TTC CAC GGT GCT ATC GAG GGC GGC AAA TTT AAT GGC GCG GCT　1056
Phe Glu Phe His Gly Ala Ile Glu Gly Gly Lys Phe Asn Gly Ala Ala
340　　345　　350
ATT TAT GAT GAT TAC GCA CAC CAC CCA ACG GAA GTA ACT GCA GTG CTC　1104
Ile Tyr Asp Asp Tyr Ala His His Pro Thr Glu Val Thr Ala Val Leu
355　　360　　365
AGC GCT GCG CGC ACC CGG GTG AAG GCC GCT GGA AAG GGC CGT GTC ATC　1152
Ser Ala Ala Arg Thr Arg Val Lys Ala Ala Gly Lys Gly Arg Val Ile
370　　375　　380
GTC GCG TTC CAA CCA CAT TTG TAC TCA CGC ACC ATG GAA TTC CAA AAG　1200
Val Ala Phe Gln Pro His Leu Tyr Ser Arg Thr Met Glu Phe Gln Lys
385　　390　　395　　400
GAG TTC GCG GAG GCA CTG TCA CTG GCA GAC GCT GCC GTG GTG CTC GAG　1248
Glu Phe Ala Glu Ala Leu Ser Leu Ala Asp Ala Ala Val Val Leu Glu
405　　410　　415
ATT TAC GGA GCG CGC GAA CAA CCG GTG GAT GGC GTG TCC TCG GAA ATC　1296
Ile Tyr Gly Ala Arg Glu Gln Pro Val Asp Gly Val Ser Ser Glu Ile
420　　425　　430
ATC ACC GAT GCG ATG ACC ATT CCA GTG GTG TAC GAA CCT AAT TTC TCT　1344
Ile Thr Asp Ala Met Thr Ile Pro Val Val Tyr Glu Pro Asn Phe Ser
435　　440　　445
GCA GTC CCA GAA CGC ATT GCA GAA ATC GCA GGA CCT AAT GAC ATC GTG　1392
Ala Val Pro Glu Arg Ile Ala Glu Ile Ala Gly Pro Asn Asp Ile Val
450　　455　　460
CTC ACC ATG GGT GCA GGT TCC GTG ACC ATG CTT GCT CCA GAA ATC CTG　1440
Leu Thr Met Gly Ala Gly Ser Val Thr Met Leu Ala Pro Glu Ile Leu
465　　470　　475　　480
GAT CAG CTG CAA AAC AAT　1458
Asp Gln Leu Gln Asn Asn
485

【００４２】配列番号：２　　＊トポロジー：直鎖状
配列の長さ：４８６　　配列の種類：タンパク質
配列の型：アミノ酸　　＊40
配列

Val Thr Thr Pro His Leu Asp Ser Ala Gln Asp Ile Asp Leu Ser Arg
1　　5　　10　　15
Val His Leu Ile Gly Ile Gly Gly Ala Gly Met Ser Gly Val Ala Arg
20　　25　　30
Ile Leu Leu Ala Arg Gly Lys Thr Val Thr Gly Ser Asp Ala Lys Asp
35　　40　　45
Ser Arg Thr Leu Leu Pro Leu Arg Ala Val Gly Ala Thr Ile Ala Val
50　　55　　60
Gly His Ala Ala Glu Asn Leu Glu Leu Ser Gly Glu Leu Pro Thr Val

13

65 70 75 80
Val Val Thr Ser Phe Ala Ala Ile Pro Gln Asp Asn Pro Glu Leu Val
85 90 95
Arg Ala Arg Glu Glu Gly Ile Pro Val Ile Arg Arg Ser Asp Leu Leu
100 105 110
Gly Glu Leu Leu Glu Gly Ser Thr Gln Val Leu Ile Ala Gly Thr His
115 120 125
Gly Lys Thr Ser Thr Thr Ser Met Ser Val Val Ala Met Gln Ala Ala
130 135 140
Gly Met Asp Pro Ser Phe Ala Ile Gly Gly Gln Leu Asn Lys Ala Gly
145 150 155 160
Thr Asn Ala His His Gly Thr Gly Glu Val Phe Ile Ala Glu Ala Asp
165 170 175
Glu Ser Asp Ala Ser Leu Leu Arg Tyr Lys Pro Asn Val Ala Val Val
180 185 190
Thr Asn Val Glu Pro Asp His Leu Asp Phe Phe Lys Thr Pro Glu Ala
195 200 205
Tyr Phe Gln Val Phe Asp Asp Phe Ala Gly Arg Ile Thr Pro Asn Gly
210 215 220
Lys Leu Val Val Cys Leu Asn Asp Pro His Ala Ala Glu Leu Gly Glu
225 230 235 240
Arg Ser Val Arg Lys Gly Ile Lys Thr Val Gly Tyr Gly Thr Ala Asp
245 250 255
Ala Val Gln Ala His Pro Glu Val Pro Ala Met Ala Thr Ile Val Asp
260 265 270
Ser Gln Val Val Ala Glu Gly Thr Arg Ala Thr Ile Asn Ile Asp Gly
275 280 285
Gln Glu Val Ser Val Ile Leu Gln Ile Pro Gly Asp His Met Val Leu
290 295 300
Asn Gly Ala Ala Ala Leu Leu Ala Gly Tyr Leu Val Gly Gly Asp Val
305 310 315 320
Asp Lys Leu Val Glu Gly Leu Ser Asp Phe Ser Gly Val Arg Arg Arg
325 330 335
Phe Glu Phe His Gly Ala Ile Glu Gly Gly Lys Phe Asn Gly Ala Ala
340 345 350
Ile Tyr Asp Asp Tyr Ala His His Pro Thr Glu Val Thr Ala Val Leu
355 360 365
Ser Ala Ala Arg Thr Arg Val Lys Ala Ala Gly Lys Gly Arg Val Ile
370 375 380
Val Ala Phe Gln Pro His Leu Tyr Ser Arg Thr Met Glu Phe Gln Lys
385 390 395 400
Glu Phe Ala Glu Ala Leu Ser Leu Ala Asp Ala Ala Val Val Leu Glu
405 410 415
Ile Tyr Gly Ala Arg Glu Gln Pro Val Asp Gly Val Ser Ser Glu Ile
420 425 430
Ile Thr Asp Ala Met Thr Ile Pro Val Val Tyr Glu Pro Asn Phe Ser
435 440 445
Ala Val Pro Glu Arg Ile Ala Glu Ile Ala Gly Pro Asn Asp Ile Val
450 455 460
Leu Thr Met Gly Ala Gly Ser Val Thr Met Leu Ala Pro Glu Ile Leu

15　　16

465　470　475　480

Asp Gln Leu Gln Asn Asn

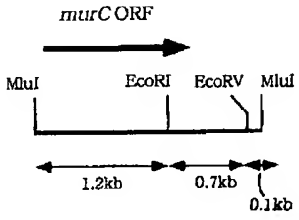
485

【図面の簡単な説明】

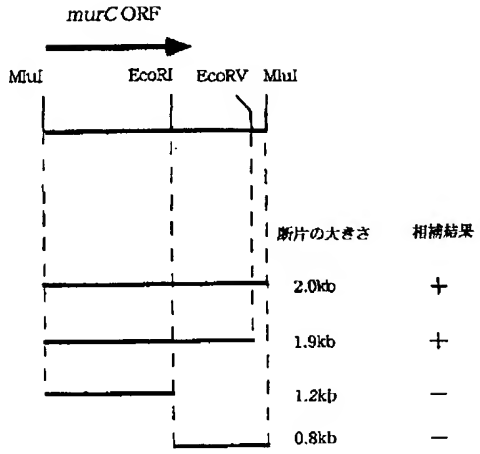
【図1】ｍｕｒＣ遺伝子を含むＤＮＡ断片の制限酵素地図（マップ）である。

＊【図2】ｍｕｒＣ遺伝子を含むＤＮＡ断片の制限酵素地図および大腸菌ｍｕｒＣ変異株を用いた相補試験結果を表す図である。

【図1】



【図2】



フロントページの続き

(72)発明者　永井　和夫
東京都豊島区池袋３の42の17

First Hit

End of Result Set

L3: Entry 17 of 17 File: DWPI Oct 15, 2002

DERWENT-ACC-NO: 1999-062655
DERWENT-WEEK: 200282

TITLE: New isolated MurC polypeptide from Staphylococcus aureus and related nucleic acid - useful in diagnosis, treatment and prevention of bacterial infections

Basic Abstract Text (1):
New isolated polypeptide (I) is: (a) is at least 70% identical to sequences (2) of 437 amino acids (fully defined in the specification) and (4) of 215 aa sequence (also fully defined) reproduced over the entire length; (b) or comprises sequences (2) or (4); or (c) encoded by recombinant nucleic acids of 1351 (1) or 660 (3) bp (also fully defined), reproduced. Also claimed are: (A) isolated nucleic acid (II) that: (i) encodes (I); (ii) is at at least 70% identical with (1) or (3) or other sequences encoding (2) and (4), over the entire length; (iii) is or comprises sequences (1) or (3); (iv) is obtained by screening a library with (1), (3) or their fragments (stringent conditions); (v) encodes the mature polypeptide expressed by the MurC gene of Staphylococcus aureus; or (vi) is complementary to any any of (i)-(v); (B) antibody (Ab) directed against (I); (C) agonist or antagonist (III) of the activity or expression of (I); (D) expression system for producing (I); (E) host cell, or derived membranes, containing this expression system; (F) computer-readable medium having sequence data for (I) and (II) stored on it.

First Hit Fwd Refs

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L12: Entry 8 of 441 File: USPT May 11, 2004

DOCUMENT-IDENTIFIER: US 6734013 B2
TITLE: Use of multivalent chimeric peptide-loaded, MHC/Ig molecules to detect, activate or suppress antigen-specific T cell-dependent immune responses

Detailed Description Text (9):
Expression vectors of the invention can be introduced into host cells using any technique known in the art. These techniques include transferrin-polycation-mediated DNA transfer, transfection with naked or encapsulated nucleic acids, liposome-mediated cellular fusion, intracellular transportation of DNA-coated latex beads, protoplast fusion, viral infection, electroporation, and calcium phosphate-mediated transfection.

First Hit Fwd Refs

Generate Collection Print

L12: Entry 13 of 441 File: USPT Apr 6, 2004

DOCUMENT-IDENTIFIER: US 6716813 B2
TITLE: Use of antimicrobial proteins and peptides for the treatment of otitis media and paranasal sinusitis

Detailed Description Text (38):
It can be envisioned that one method of administering the lysozyme, lactoferrin, alpha defensins, and beta defensins uses expression vectors which express these proteins. The expression vectors may be targeted to the tissue or cell which is infected or which is near the infected cells. The vectors may be any vectors known to one of skill in the art including but not limited to: viral vectors, plasmid vectors, and naked DNA. Expression from these vectors may be constitutive or may be under the control of a specific promoter, such as a eukaryotic promoter, or an inducible promoter.

First Hit Fwd Refs

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L12: Entry 60 of 441 File: USPT Oct 21, 2003

DOCUMENT-IDENTIFIER: US 6635249 B1
TITLE: Methods for treating congestive heart failure

Detailed Description Text (16):
The expression vector may be administered as naked DNA mixed with or conjugated to an agent to enhance the entry of the DNA into cells, e.g., a cationic lipid such as Lipofectin.TM., Lipofectamine.TM. (Gibco/BRL, Bethesda, Md.), DOTAP.TM. (Boeringer-Mannheim, Indianapolis, Ind.) or analogous compounds, liposomes, or an antibody that targets the DNA to a particular type of cell, e.g., a cardiomyocyte or an endothelial cell. The method of administration may be any of those described in the Therapy section above. In particular, DNA for somatic gene therapy has been successfully delivered to the heart by intravenous injection, cardiac perfusion, and direct injection into the myocardium (e.g., see Losordo et al., Circulation 98:2800-2804, 1998; Lin et al., Hypertension 33:219-224, 1999; Labhasetwar et al., J. Pharm. Sci. 87:1347-1350, 1998; Yayama et al., Hypertension 31:1104-1110, 1998). The therapeutic DNA is administered such that it enters the patient's cells and is expressed, and the vector-encoded therapeutic polypeptide binds to and activates cardiomyocyte ErbB receptors.

First Hit Fwd Refs

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L12: Entry 97 of 441 File: USPT Jul 29, 2003

DOCUMENT-IDENTIFIER: US 6599692 B1
TITLE: Functional genomics using zinc finger proteins

Detailed Description Text (30):
"Administering" an expression vector, nucleic acid, zinc finger protein, or a delivery vehicle to a cell comprises transducing, transfecting, electroporating, tanslocating, fusing, phagocytosing, or biolistic methods, etc., i.e., any means by which a protein or nucleic acid can be transported across a cell membrane and preferably into the nucleus of a cell, including administration of naked DNA.

Detailed Description Text (45):
An "expression vector" is a nucleic acid construct, generated recombinantly or synthetically, with a series of specified nucleic acid elements that permit transcription of a particular nucleic acid in a host cell, and optionally, integration or replication of the expression vector in a host cell. The expression vector can be part of a plasmid, virus, or nucleic acid fragment, of viral or non-viral origin. Typically, the expression vector includes an "expression cassette," which comprises a nucleic acid to be transcribed operably linked to a promoter. The term expression vector also encompasses naked DNA operably linked to a promoter.

Detailed Description Text (118):
Expression vectors can be delivered in vivo by administration to an individual subject, typically by systemic administration (e.g., intravenous, intraperitoneal, intramuscular, subdermal, or intracranial infusion) or topical application, as described below. Alternatively, naked DNA can be administered. Alternatively, vectors can be delivered to cells ex vivo, such as cells explanted from an individual subject (e.g., lymphocytes, bone marrow aspirates, tissue biopsy) or universal donor hematopoietic stem cells, followed by reimplantation of the cells into a patient, usually after selection for cells which have incorporated the vector.

sp Q9ZLL2 MURC_HELPJ **UDP-N-acetylmuramate--L-alanine ligase (EC 6.3.2.8) (UDP-N-acetylmuramoyl-L-alanine synthetase) [MURC] [Helicobacter pylori J99 (Campylobacter pylori J99)]** 449 AA align

Score = 849 bits (2194), Expect = 0.0
Identities = 428/449 (95%), Positives = 428/449 (95%)

```
Query: 1   MLETPKVLLKNLQDCKIHFXXXXXXXXSGLAKYLKAQGAKISGSDIAISPSVKYLKALGV 60
           MLETPKVLLKNLQDCKIHF        SGLAKYLKAQGAKISGSDIAISPSVKYLKALGV
Sbjct: 1   MLETPKVLLKNLQDCKIHFIGIGGIGISGLAKYLKAQGAKISGSDIAISPSVKYLKALGV 60

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Sbjct: 61  EINIPHDPKAINHQDVIIHSAIIKEDNTEIQRAKELEIPILSRKDALYSILKDKRVFSVC 120

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Query: 181 AIVPNTEPEHLEHYDHDLERFFFAYKYFLDHAQKRVIYKEDPFLKNYSKDAIVLEKKDIY 240
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Sbjct: 181 AIVPNTEPEHLEHYDHDLERFFFAYKYFLDHAQKRVIYKEDPFLKNYSKDAIVLEKKDIY 240

Query: 241 NIQYILKDGEPYTSFELKNLGAFLVWGLGEHNATNASLAILSALDXXXXXXXXXXXXXFK 300
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Query: 421 NDNVVETIEKGFVIGFGAGDITYQLRGEM 449
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Sbjct: 421 NDNVVETIEKGFVIGFGAGDITYQLRGEM 449
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NiceProt View of Swiss-Prot: Q9ZLL2

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[Entry info] [Name and origin] [References] [Comments] [Cross-references] [Keywords] [Features] [Sequence] [Tools]

Note: most headings are clickable, even if they don't appear as links. They link to the user manual or other documents.

Entry information

Entry name	**MURC_HELPJ**
Primary accession number	**Q9ZLL2**
Secondary accession numbers	None
Entered in Swiss-Prot in	Release 39, May 2000
Sequence was last modified in	Release 39, May 2000
Annotations were last modified in	Release 44, June 2004

Name and origin of the protein

Protein name	**UDP-N-acetylmuramate--L-alanine ligase**
Synonyms	**EC 6.3.2.8** **UDP-N-acetylmuramoyl-L-alanine synthetase**
Gene name	**MURC** or **JHP0567**
From	Helicobacter pylori J99 (Campylobacter pylori J99) [TaxID: 85963]
Taxonomy	Bacteria; Proteobacteria; Epsilonproteobacteria; Campylobacterales; Helicobacteraceae; Helicobacter.

References

[1] SEQUENCE FROM NUCLEIC ACID.
MEDLINE=99120557; PubMed=9923682; [NCBI, ExPASy, EBI, Israel, Japan]
Alm R.A., Ling L.-S.L., Moir D.T., King B.L., Brown E.D., Doig P.C., Smith D.R., Noonan B., Guild B.C., deJonge B.L., Carmel G., Tummino P.J., Caruso A., Uria-Nickelsen M., Mills D.M., Ives C., Gibson R., Merberg D., Mills S.D., Jiang Q., Taylor D.E., Vovis G.F., Trust T.J.;
"Genomic sequence comparison of two unrelated isolates of the human gastric pathogen Helicobacter pylori.";
Nature 397:176-180(1999).

Comments

- ***FUNCTION***: Cell wall formation *(By similarity)*.
- ***CATALYTIC ACTIVITY***: ATP + UDP-N-acetylmuramate + L-alanine = ADP + phosphate + UDP-N-acetylmuramoyl-L-alanine.
- ***PATHWAY***: Peptidoglycan biosynthesis.

- ***SUBCELLULAR LOCATION***: Cytoplasmic *(Probable)*.
- ***SIMILARITY***: Belongs to the murCDEF family.

Copyright

Cross-references

EMBL	AE001489; AAD06138.1; -.[EMBL / GenBank / DDBJ] [CoDingSequence]
PIR	B71917; B71917.
HSSP	P45066; 1GQQ. [HSSP ENTRY / PDB]
CMR	Q9ZLL2; JHP0567.
HAMAP	MF_00046; -; 1. PBIL [Family / Alignment / Tree]
InterPro	IPR005758; MurC. IPR000713; Mur_ligase. IPR004101; Mur_ligase_C. Graphical view of domain structure.
Pfam	PF01225; Mur_ligase; 1. PF02875; Mur_ligase_C; 1. Pfam graphical view of domain structure.
TIGRFAMs	TIGR01082; murC; 1.
ProDom	[Domain structure / List of seq. sharing at least 1 domain]
HOBACGEN	[Family / Alignment / Tree]
BLOCKS	Q9ZLL2.
ProtoNet	Q9ZLL2.
ProtoMap	Q9ZLL2.
PRESAGE	Q9ZLL2.
DIP	Q9ZLL2.
ModBase	Q9ZLL2.
SMR	Q9ZLL2; 4EDD7CCF8AF5D74F.
SWISS-2DPAGE	Get region on 2D PAGE.
UniRef	View cluster of proteins with at least 50% / 90% identity.

Keywords

Peptidoglycan synthesis; **Cell wall**; **Cell division**; **Ligase**; **ATP-binding**; **Complete proteome**.

Features


Feature table viewer

Key	From	To	Length	Description
NP_BIND	121	127	7	ATP (*Potential*).

Sequence information

Length: **449 AA** Molecular weight: **50906 Da** CRC64: **4EDD7CCF8AF5D74F** [This is a checksum on the sequence]

```
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         |          |          |          |          |          |
```

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          |           |           |           |           |           |
EINIPHDPKA INHQDVIIHS AIIKEDNTEI QRAKELEIPI LSRKDALYSI LKDKRVFSVC

        130         140         150         160         170         180
          |           |           |           |           |           |
GAHGKSSITA MLSAICPAFG AIIGAHSKEF DSNVRESADM SLVFEADESD SSFLFSNPFC

        190         200         210         220         230         240
          |           |           |           |           |           |
AIVPNTEPEH LEHYDHDLER FFFAYKYFLD HAQKRVIYKE DPFLKNYSKD AIVLEKKDIY

        250         260         270         280         290         300
          |           |           |           |           |           |
NIQYILKDGE PYTSFELKNL GAFLVWGLGE HNATNASLAI LSALDELNLE EIRNNLLNFK

        310         320         330         340         350         360
          |           |           |           |           |           |
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          |           |           |           |           |           |
DNLEEFKKCF LEHCDRLIIL PVYSASEVKR DIDLKAHFKH YNPTFIDRVR KKGDFLELLV

        430         440
          |           |
NDNVVETIEK GFVIGFGAGD ITYQLRGEM
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Q9ZLL2 in FASTA format

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BLAST submission on ExPASy/SIB or at NCBI (USA)

Sequence analysis tools: ProtParam, ProtScale, Compute pI/Mw, PeptideMass, PeptideCutter, Dotlet (Java)

ScanProsite, MotifScan

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L3: Entry 1 of 17 File: PGPB Mar 18, 2004

DOCUMENT-IDENTIFIER: US 20040052799 A1
TITLE: Nucleic acid and amino acid sequences relating to Helicobacter pylori for diagnostics and therapeutics

CLAIMS:

182. An isolated nucleic acid comprising a nucleotide sequence encoding an H. pylori pylori murC polypeptide or a fragment thereof, said nucleic acid comprising the nucleotide sequence of SEQ ID NO: 2845.

188. A purified H. pylori murC polypeptide or a fragment thereof, wherein said polypeptide comprises the amino acid sequence of SEQ ID NO: 7607.

First Hit

L3: Entry 1 of 17 File: PGPB Mar 18, 2004

PGPUB-DOCUMENT-NUMBER: 20040052799
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20040052799 A1

TITLE: Nucleic acid and amino acid sequences relating to Helicobacter pylori for diagnostics and therapeutics

PUBLICATION-DATE: March 18, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Smith, Douglas	Gloucester	MA	US	
Alm, Richard A.	Ashland	MA	US	
Berglindh, O. Thomas	Uppsala	MA	SE	
Mellgard, Bjorn L.	Goteborg	MA	SE	
Thomas, Steven R.	Mission	MA	CA	
Brown, Eric	Cambridge	MA	US	
Moir, Donald T.	Lexington	MA	US	
Ling, Lo See	Arlington	MA	US	
Carmel, Gilles	Waltham	MA	US	
Guild, Braydon C.	Concord	MA	US	
Doig, Peter C.	Acton		US	
Kabok, Zita	Natick		US	
Castriotta, Lillian Marie	Newton		US	

ASSIGNEE-INFORMATION:

NAME	CITY	STATE	COUNTRY	TYPE CODE
Astra Aktiebolag				02

APPL-NO: 10/ 335977 [PALM]
DATE FILED: December 30, 2002

RELATED-US-APPL-DATA:
Application 10/335977 is a continuation-of US application 08/993002, filed December 17, 1997, ABANDONED
Application 08/993002 is a continuation-in-part-of US application 08/821931, filed March 21, 1997, ABANDONED
Application 08/821931 is a continuation-of US application 08/761184, filed December 5, 1996, ABANDONED

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
WO	PCT/US97/19575	1997WO-PCT/US97/19575	October 28, 1997
WO	PCT/US97/22104	1997WO-PCT/US97/22104	December 5, 1997
WO	PCT/US96/18542	1996WO-PCT/US96/18542	November 15, 1996

INT-CL: [07] A61 K 39/00, A61 K 39/38

US-CL-PUBLISHED: 424/184.1
US-CL-CURRENT: 424/184.1

REPRESENTATIVE-FIGURES: NONE

ABSTRACT:

Recombinant or substantially pure preparations of H. pylori polypeptides are described. The nucleic acids encoding the polypeptides also are described. The H. pylori polypeptides are useful for diagnostics and vaccine compositions.

RELATED APPLICATIONS

[0001] The contents, including the Sequence Listings and Figures, of each of the following related applications listed by serial number and filing date is incorporated herein by reference.

[0002] This application is a continuation-in-part of U.S. Ser. No. 08/821,931, filed Mar. 21, 1997, which is a continuation of U.S. Ser. No. 08/761,184, filed Dec. 6, 1996 now abandoned, which is a continuation-in-part of U.S. Ser. No. 08/759,739, filed Dec. 6, 1996, which is a continuation-in-part of U.S. Ser. No. 08/736,791, filed Oct. 25, 1996, which is a continuation-in-part of U.S. Ser. No. 08/739,150, filed Oct. 28, 1996, which is a continuation-in-part of 08/660,742, filed Jun. 6, 1996, which is a continuation-in-part of U.S. Ser. No. 08/630,405, filed Apr. 1, 1996, which is a continuation-in-part of U.S. Ser. No. 08/561,469, filed Nov. 17, 1995, which is a continuation-in-part of U.S. Ser. No. 08/487,032, filed Jun. 7, 1995. This application also claims priority to PCT application PCT/US97/19575, filed Oct. 28, 1997, and PCT application PCT/US96/18542, filed Nov. 15, 1996. This application is also a continuation-in-part of U.S. Ser. No. 08/625,431 filed Mar. 26, 1996 which is a continuation-in-part of U.S. Ser. No. 08/621,425, filed Mar. 25, 1996, which is a continuation-in-part of U.S. Ser. No. 08/561,469, filed Nov. 17, 1995, which is a continuation-in-part of U.S. Ser. No. 08/487,032, filed Jun. 7, 1995. This application is also a continuation-in-part of U.S. Ser. No. 08/824,132, filed Mar. 27, 1997, which is a continuation-in-part of U.S. Ser. No. 08/761,318, filed Dec. 6, 1996, which is a continuation-in-part of U.S. Ser. No. 08/738,859, filed Oct. 28, 1996 now abandoned, which is a continuation-in-part of U.S. Ser. No. 08/625,811, filed Mar. 29, 1996. This application is also a continuation-in-part of U.S. Ser. No. 08/769,224, filed Dec. 6, 1996, which is a continuation-in-part of U.S. Ser. No. 08/736,905, filed Oct. 25, 1996 now abandoned, which is a continuation-in-part of U.S. Ser. No. 08/758,731, filed Apr. 2, 1996. This application is also a continuation-in-part of U.S. Ser. No. 08/823,745, filed Mar. 25, 1997, which is a continuation-in-part of U.S. Ser. No. 08/759,625, filed Dec. 5, 1996. This application also claims priority to a PCT application, number not yet available, filed Dec. 5, 1997, (Atty. Docket No. GTN-011CP2PC). This application is also a continuation-in-part of U.S. Ser. No. 08/761,066, filed Dec. 5, 1996. This application is also a continuation-in-part of U.S. Ser. No. 08/891,928, filed Jul. 14, 1997. This application is also a continuation-in-part of U.S. Ser. No. 08/892,020, filed Jul. 14, 1997.

[0003] It should be understood that all of the Sequence Listings and Figures of all of the aforementioned applications are considered to be part of the contents which are incorporated by reference herein. For example, FIG. 1 (pages 1-1199), which contains H. pylori genomic DNA, of U.S. Ser. No. 08/621,425, filed Mar. 25, 1996 is incorporated herein by reference. FIG. 1 (pages 1-1199), which contains H. pylori genomic DNA, and FIGS. 2-809 and FIG. 810 (pages 908-2700), which contain H. pylori

amino acid sequences, of U.S. Ser. No. 08/625,431, filed Mar. 26, 1996 also are incorporated herein by reference.

US 104645800BP1

Creation date: 06-24-2004
Indexing Officer: SUK - SOPHY UK
Team: OIPEBackFileIndexing
Dossier: 10464580

Legal Date: 06-25-2004

No.	Doccode	Number of pages
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3	892	1
4	FOR	22
5	FWCLM	1
6	SRFW	1

Total number of pages: 37

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